Vulnerability of Selected Native and Invasive Woody Species to Streamflow Variability in Western Cape Fynbos Riparian Ecotones

by

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Declaration

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Summary

Riparian ecosystems of the Mediterranean south-western Cape region are projected to experience significant decreases in streamflow due to climate change and increased demands for water associated with human demographic trends and increasing living standards. Aggravating this problem are woody invasive alien plants, such as *Acacia mearnsii*, whose impacts, including those on catchment water yields, have justified extensive eradication programmes such as Working for Water (WfW). WfW has been highly successful in managing invasive alien species in some areas, while at the same time enhancing the quality of life of poor South Africans. Unfortunately, the managers of these eradication initiatives often experience a lack of information on the species- and site-specific ecological properties that may aid in prioritising sites more prone to invasion, essentially inhibiting accurate management output. Knowledge, such as that associated with the possible future distribution of invasive species in a changing environment, may help to optimise eradication initiatives. I therefore determined whether woody plants portray different physiological (vulnerability to cavitation) and wood anatomical traits (wood density, vessel resistance to implosion, vessel lumen and wall diameters) across three prominent riparian zones in the south-western Cape that each differ in streamflow quantity – to gain a mechanistic understanding of how woody species, especially invasive species, adapt their hydraulic strategy across this proxy for water availability. Using factorial ANOVA's, to distinguish any differences in plant physiological and wood anatomical responses to water availability within and between species, showed *Acacia mearnsii* having consistently higher drought-tolerance (lower P_{50} values and denser wood) compared to native species under reduced water availability. These results supported the significant variation in drought-tolerance strategies that exist within and between taxonomically different species across different environments. Water availability thus has a strong selective effect on functional traits of species; however, minimum water potentials were more useful in describing *in situ* hydrological conditions than streamflow. Additionally, a non-causal relationship between wood anatomical traits and drought-induced cavitation was observed. Therefore, using only wood anatomical measurements to assess drought-tolerance of species might not be accurate across all species. This mechanistic approach to assess the invasive potential of species under projected drier conditions has great practical value. It can be used to improve species selection for restoration initiatives, and is of great value for future use in prioritizing eradication programmes.

Samevatting

Rivieroewer ekosisteme in die Mediterreense Suidwes-Kaap streek kan groot afnames in stroomvloei verwag, wat meestal toegeskryf kan word aan klimaatsverandering en die verhoogde aanvraag na water geassosieerd met stygende populasiegrootte. Uitheemse indringerplante soos *Acacia mearnsii* vererger hierdie probleem. Laasgenoemde spesies plaas groot druk op opvanggebiede se water opbrengs, en vorm grootliks die rede waarom uitroeiing programme soos Werk vir Water (WfW) gestig is. WfW het al groot suksesse behaal in die bestuur van uitheemse indringerplante, en terselfdertyd die lewenskwaliteit van arm Suid-Afrikaners verhoog. Ongelukkig sukkel hierdie indringer uitroeiing inisiatiewe telkens met ongenoegsame bewyse wat kan help om leiding te verskaf by die prioritisering van areas meer geneig tot indringing. Die gevolg is dat onvoldoende bestuurs besluite gemaak kan word. Gevolglik sal kennis geassosieerd met die moontlike toekomstige verspreiding van hierdie indringerplant in 'n veranderlike omgewing help om uitroeiing programme meer akkuraat te stel en gevolglik waardevol wees t.o.v. prioritisering van kwesbare areas. Die doel van hierdie studie was om die kwesbaarheid van sleutel inheemse houtagtige rivier spesies en veral die dreigende indringerplant *Acacia mearnsii*, te bestudeer in terme van verlaagde stroomvloei soos voorspel vir die Wes-Kaap fynbos ekosisteem. Deur te bepaal of houtagtige plante verskillende fisiologiese (kwesbaarheid teen xileem blokkasie) en anatomiese (hout digtheid, vat weerstandbiedendheid teen inmekaarkolking, vat holte en wand deursnee) hidrologiese eienskappe tentoonstel gemeet tussen drie prominente rivieroewer sones in die suidwes Kaap wat variëer in stroomvloei kwantiteit, verskaf 'n meganistiese begrip van hoe spesies, veral die indringerplante, hul hidrologiese strategieë ten opsigte van droogtes kan aanpas. Faktoriële ANOVAS's tussen spesies en liggings het getoon dat beide plant fisiologiese en hout anatomiese data beduidend variëer tussen taksonomies verskillende spesies. Veral *Acacia mearnsii* het deurgaans laer P_{50} waardes asook hoër houtdigtheid getoon, 'n teken van hierdie spesies se verhoogde droogte toleransie in vergelyking met die inheemse spesies. Wat beduidend is is dat spesies-spesifieke lewensstrategieë ten opsigte van droogte toleransie in hierdie rivieroewer sones voorkom. Resultate het bevestig dat water beskikbaarheid 'n groot rol speel in die seleksie van funksionele karaktereienskappe in plante, maar, dat minimum water potensiaal meer akkuraat was om *in situ* hidrologiese toestande te beskryf as wat stroomvloei kon. Die verhouding tussen plant fisiologiese en hout anatomiese karaktereienskappe was indirek as gevolg van spesies-spesifieke lewensstrategieë. Gevolglik sal die allenige gebruik van hout anatomiese eienskappe om droogte toleransie in plante te assesseer onakkuraat wees omdat daar 'n swak verhouding tussen hout anatomie en droogte-geïnduseerde xileem blokkasie bestaan. 'n Meganistiese benadering om die indringing potensiaal van spesies onder voorspelde droër kondisies te assesseer, asook om spesies seleksie vir restourasie doeleindes op te skerp, is prakties uitvoerbaar, en van groot waarde vir toekomstige gebruik in uitroeiing en restourasie inisiatiewe.

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Literature Review:

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Summary

Riparian zones form the interface between freshwater environments and the larger terrestrial landscape, and include river banks and the margins of lakes and estuaries. These spatially and temporally diverse zones often support a unique biotic community, both structurally and compositionally delineating itself from the larger surrounding landscape. Riparian zones function as an important buffer zone that regulates material flows between terrestrial and aquatic environments. The nestedness of riparian zones within the landscape suggests an integrative approach to management of riparian biodiversity as an integral part of the 'water resource' (Ashton, 2007). This review focuses on the structure and function of riparian zones in the Mediterranean climate south-western Cape region, to assess the responses of riparian woody species to changes in water resources. One of the main threats to functioning of riparian zones is the invasion of woody alien invasive species. While the impacts of alien invasive species on runoff have been well documented, the implications for community structure and composition with future changes in climatic conditions have received less attention. The implications for eradication initiatives are also put into this context. The value of a mechanistic approach to restoration ecology is explored through looking at physiological and anatomical plant-water relations as indicators of vulnerability to drought, and thus indicators of possible future distributions of native and invasive species. I review this approach as a tool for prioritizing restoration initiatives, and suggest key questions that will be addressed in the thesis that throw light on the future integrity of riparian zones, and thus water resources in a changing environment.

1.1. Water and South Africa

South Africa is a water scarce country with an average rainfall of around 450 mm per annum compared to a global average of 860 mm. With only 8.6% of rainfall available as surface water, one of the lowest conversion ratios in the world, it is evident that freshwater is a limiting resource that will severely inhibit future socio-economic growth (Walmsley et al., 1999). Demographic trends suggest that there will be approximately 60-75 million people in South Africa around 2025 (Davies and Day, 1998) compared to the current 47 million. Consequently, there will be strong pressure on water resources, coming from both the agricultural (irrigation) and urban development (domestic) sectors to provide in the basic needs for the projected population increase. The tragedy is that most of South Africa's surface and groundwater resources have already been developed (as reviewed in Otieno and Ochieng, 2004). It is therefore important that strategies for sustainable water management strategies must be implemented to alleviate unnecessary strains on water resources, and so prevent dire water scarcity in South Africa (Walmsley et al., 1999; Otieno and Ochieng, 2004; Ashton, 2007). South African river systems are particularly threatened as less than a third of the main rivers are seen as ecologically intact (Nel et al., 2007). Eighty five percent of all water used in South Africa comes from these main rivers, which is why greater emphasis needs to be put on riparian biodiversity conservation as a method of ensuring sustainability of ecosystem services (Ashton, 2007). Protected areas, where river conservation is more effective, are especially being assessed in terms of increasing their extent to aid in addressing this challenge (Nel et al., 2009).

Possibly contributing to this environmental stress is global climate change, a reality that could further severely threaten the already limited water supply (to name only one aspect) in South Africa (Meadows, 2006). According to 2050 climate change scenario models roughly 16% of South Africa (195 000 km^2) is likely to experience an increase in water shortage (Schulze et al., 2001). Predictions of climatic change in the Mediterranean south-western Cape district suggest overall drier conditions (New, 2002). Consequently riparian vegetation could experience increased water-stress, especially where the water table of perennial rivers drop significantly. This would directly impact riparian zone biodiversity and thus lead to reduced water quality (Midgley et al., 2003). Conversely, more intense storms are expected, which may increase flash floods, impact riparian geomorphology and modify riparian plant communities.

Modelling of global warming trends in selected south-western Cape rivers indicate that some may lose between 14% and 32% of their streamflow (New, 2002). The Breede

River, the largest river in the Western Cape, is predicted to experience a significant decrease in mean annual run-off due to unfavourable climatic changes (Steynor et al., 2009). Steynor et al. (2009) projected this decrease without incorporating increased water extraction from agriculture and urban sectors. Taking into account the predicted increased demands on water resources due to demographic and economic growth, it becomes clear why climate change will increase stress on the communities already vulnerable to drought (Mukheibir and Sparks, 2005). Therefore, climate change, in synergy with population growth in the south-western Cape region, will lead to water demand overwhelming the actual supply – leaving this region prone to environmental deterioration which may eventually result in negative socio-economic consequences (Davies and Day, 1998; New, 2002).

Hydrological systems in southern Africa, especially the south-western Cape, are highly vulnerable given future predictions of climatic change and there is justifiable concern about matters pertaining to water resource management. The projected declines in available water resources become even more alarming when the threat of invasive alien species is considered.

1.2. The Impact of Invasive Alien Species on Water Resources and Riparian Ecology

Invasive alien plants (IAP) are a global problem. In South Africa, species within the genera *Pinus* and *Eucalyptus,* which are now problematic invaders, were introduced to supply a growing demand for wood related products. The invasive nature of these tree species is explained by a variety of factors including their life-history (fast growing, copious seed production, adapted to fire), economic incentives for introduction (used for a wide variety of products, creating a healthy economic market for trading purposes) and predator release (few natural enemies that can impact the quality of the wood) (Richardson, 1998). *Acacia* spp. were first introduced to assist in combating soil erosion, and to provide fuel wood and various related wood products. Yet, despite their economic value, the enormous impact these species have on biodiversity and water resource usage was recognised early on (Bosch and Hewlett, 1982). Subsequently, scientists began to investigate the possible effects that these invading timber plantations may have on water resources, especially streamflow reductions, in South Africa (Bosch and Hewlett, 1982; Le Maitre et al., 1996; Scott et al., 1998; Enright, 2000; Dye et al., 2001; Le Maitre et al., 2002; Dye and Jarmain, 2004).

Timber plantations in South Africa are usually planted in higher rainfall areas with an annual rainfall between 800-1000mm (Scott et al., 1998), ensuring ample water supply for

cultivating dense stands of trees. However, in both high and low rainfall areas forestry plantations have been shown to result in a reduction in streamflow (Bosch and Hewlett, 1982; Scott et al., 1998); specifically dense stands of *Acacia mearnsii* in riparian zones have been shown to exhibit high amounts of evaporative loss (Dye et al., 2001; Dye and Jarmain, 2004). Clearing plantations in riparian zones can increase the streamflow significantly, more so than when clearing plantations from up-slope catchment areas (Scott et al., 1998). As a result, forestry policy today does not allow exotic timber to be planted in the riparian zone of the catchment. However, the problem of runaway invasion of certain species, especially *Acacia mearnsii* (Black Wattle), into the riparian zones still exists. South African riparian zones support some of the densest stands of these runaway invasives (Le Maitre et al., 2002; Dye and Jarmain, 2004). This is especially true for the Western Cape which exhibits the highest degree of *Acacia mearnsii* invasion, and subsequently also high streamflow reductions (Enright, 2000). Models predicting future consequences of a lack of management of IAP and water resources in the Western Cape indicate that if the current growth of alien plants goes unchecked from the present state for a hundred years, some species might invade up to 62.4% of the catchment area with about 87 million cubic meters of water lost due to this invasion (Le Maitre et al., 1996). This translates to a 34% decrease in annual water availability for the City of Cape Town municipal area (Le Maitre et al., 1996). Riparian vegetation acts as an ecosystem service provider by providing a buffer against floods (and thus erosion), nutrient recycling as well as habitat for an array of biota (Tabacchi et al., 1998). Thus, by managing the integrity of the riparian zone through removing invasive woody biomass, river systems may continue to provide quality and quantity water and as a result sustain crucial ecosystem services. This would also help to lessen the possible synergistic effects from climate change and population increases faced by hydrological systems in the south-western Cape, especially when we do not know if climate change will further increase the invasion success of trees like *Acacia mearnsii*.

1.3. Invasion Success of IAPs in a Changing Environment

An important question has been raised concerning the future invasion success of alien plants under global climate change – could IAP become more invasive, and thus be even more competitive, or could their invasion success be reduced (Dukes and Mooney, 1999). Plants species are known to respond positively to increased CO_2 levels (increased water-use efficiency at the leaf level, especially pronounced in drier areas), and are thus able to

capitalise on aspects of climate change (Smith et al., 2000). A study done on islands in the Mediterranean Basin showed how broadleaved temperate invasive tree species had the ability to become more invasive under global warming projections (Gritti et al., 2006). Climate change could thus dramatically increase the future distribution of an IAP, as was predicted for *Acacia nilotica* invasion in Australia (Kriticos et al., 2003). In general, IAPs are perceived to become more invasive under global climate change due to specific advantageous life-history strategies (Dukes and Mooney, 1999). However, much research must still be conducted to gain more knowledge of specific plant traits that allows a species to become more invasive (Pysek et al., 1995), for instance its physiological traits.

Bradford et al. (2007) modelled how IAP in riparian zones can outcompete co-occurring native species with regards to N and light availability. They observed IAP to have higher biomass and higher N content than the native riparian species. Resource competition can thus be seen as key mechanism for competitive advantage in certain habitats. Cleverly et al. (1997) illustrated how *Tamarix ramosissima*, an invader of floodplains and riparian zones in south-western USA, showed higher drought-tolerance when compared to native co-occurring species and may therefore have the ability to dominate its invaded habitat during drought-stressed conditions.

Given the current water resource dilemma in South Africa and the Mediterranean south-western Cape, it is essential to compare ecological strategies pertaining to drought-stress responses for IAP and the important native riparian species it displaces. It would help assess the performance of IAP under reduced water availability scenarios, and could be a useful screening tool for future distribution of IAP (Richardson and Van Wilgen, 2004).

1.4. Response to the Problem and Future Needs and Shortcomings

In response to South Africa signing the Convention on Biological Diversity's 2010 target – to significantly reduce the loss of biodiversity in the country by 2010 – government and scientific agencies realized the need to improve conservation efforts to help lessen the effect of IAPs on biodiversity and thus also ensuring sustainability in the water sector. Consequently more thought was given towards optimising conservation initiatives such as the Working for Water (WfW) programme that commenced in 1995 (Van Wilgen et al., 1998). Through this project, eradication of IAPs from the riparian zones was initiated to maintain and restore ecosystem services, and to improve the livelihood of impoverished people by providing jobs. A challenge this programme currently faces is the variability of climate

trends: models may not always accurately project the occurrence of drought conditions for a certain area, thus leading to uncertainty in terms of prioritising areas for clearing. Even though we may be able to broadly estimate what the possible impact on water resources may be, there might always be the chance that we spend valuable money on species that may be less vulnerable to the proposed changes in habitat, and on the wrong areas (Van Wilgen et al., 1998). It is thus important for ecologists to investigate which IAP pose the greatest risk to the native riparian vegetation, as this can help with a more focussed allocation of funds and ultimately more effective conservation management (Chornesky and Randall, 2003; Richardson and Van Wilgen, 2004). Also, chances are that some (currently) non-invasive alien plants may become more invasive as climate change progresses (i.e. the opposite may also be true) (Dukes and Mooney, 1999; Richardson and Van Wilgen, 2004). As an example, the question could be asked whether in a drier and hotter future, *Acacia mearnsii* would be able to survive and outcompete native species in river systems, with detrimental impacts on riparian structure, biodiversity and functioning.

This insight implies a need for more research in riparian restoration. Many restoration initiatives may fall short of delivering their intended ecological service, due to a lack of scientific evidence that the proposed inputs will deliver feasible outputs. This will lead to increased risk taking in decision making, and could prove very costly (Palmer and Filoso, 2009). A review by Holmes et al. (2005) highlighted the need to re-introduce native trees after clearing to optimize post-clearing maintenance and so prohibit re-invasion. However, they came to the conclusion that the feasibility of such research may be obstructed by a lack of evidence that the cost of the restoration will match the benefits. As active restoration is shown to be important for mitigating riparian zones after invasion (Reinecke et al., 2008), emphasis must be put on cost efficiency through sound scientific decision making.

A study done on vulnerability of fynbos riparian vegetation towards potential reductions in streamflow (Swift et al., 2008) has shown the value of this type of research. By using an ecophysiological approach, this study showed that key riparian tree species growing along low flow streams are more drought-tolerant than those growing along higher flow streams, even though it has been observed that riparian plants are not directly influenced by the amount of streamside water (Dawson and Ehleringer, 1991). Results like these can be used to a) pin point certain native species that are more tolerant or adapted to a certain environmental gradient, and b) prioritize areas where species will be more affected by a sudden drop in streamflow. This could enable decision makers to have a more solid scientific base when deciding what species to re-introduce where, as some species might not survive in the cleared landscape, especially with lower water availability. Invasive species'

susceptibility to climate change, especially drought, could also be measured this way and will have the potential to provide insight into their future impacts and how they compete with native species. Fully understanding plant-water relations on the physiological and anatomical level is therefore important in defining these key drought-tolerant species.

1.5. A Physiological and Anatomical Approach to Understand the Impact of Changes in Water Supply on Plant Individuals and Populations

1.5.1. Plant-water relations

To fully investigate the mechanisms that drive water-related ecosystem services in riparian zones, we first need to focus on an understanding of the broad principles that underlie plant-water relations. As water constitutes about 85-90% of all growing tissue in plants (Grace, 1997), it is crucial that plants have a safe and sustainable mechanism for obtaining and transporting water. Plant water usage characteristics, and ultimately the diverse ecological range occupation of plants, can be influenced by the relationship between relative water content and its water potential (Höfler-Thoday principle) (Grace, 1997). To illustrate this relationship, Grace (1997) compared two species – one mesic and the other xeric – and found that the xeric species was better able to maintain positive turgor at lower water potentials, leading to increased drought-tolerance and lending insight to their competitive advantage in water limited situations.

Water transport in plants can be explained through the soil-plant-atmosphere continuum. Through the process of photosynthesis leaves transpire, exchanging water for carbon dioxide. Thus, a lower water potential (more negative water potential) occurs at the leaf level, the site of carbon-water exchange, while the roots will experience a more positive water potential – in effect creating a pressure gradient (Grace, 1997). Water will always move from a higher to a lower water potential. Xylem water potentials are thus negative because of suction pressure. Plant water potential in the moist soil is near zero (Grace, 1997). For movement of water to occur, the pressure at leaf level has to be more negative. Subsequently water is passively (requiring no metabolic input) pulled up vertically from the soil due to the cohesion-tension mechanism of xylem water transport (as reviewed in Hacke and Sperry, 2001). This mechanism allows the water to move along the xylem through the aforementioned pressure gradient created by the rate of transpiration.

Once at the transpiring surface, there exist two strategies plants can adopt to regulate water movement. Firstly, plants that experience very low water potentials (difficulty in accessing soil water or high vapour pressure deficits) might reduce their stomatal conductance in an attempt to prevent water-stress and possible die-back (Lambers et al., 1998, Linton et al., 1998). These plants are known as isohydric plants (McDowell et al., 2008). Secondly there are plants that are able to transpire under low water potentials known as anisohydric plants (McDowell et al., 2008). However, both isohydric and anisohydric strategies have severe draw-backs under different drought conditions. When intense drought occurs, anisohydric plants might not be able to tolerate the sudden drop in water potentials because of their inability to close their stomata. On the other hand, isohydric plants might suffer carbon starvation under extended drought as stomatal closure over long periods will lead to reduced carbon assimilation, inhibiting plant metabolism and growth (McDowell et al., 2008). Identifying which strategy a plant will use in an environment can be insightful pertaining to understanding the future survival and distribution of species under projected increases in drought conditions brought on by global climate change. One way of determining whether plants are anisohydric or isohydric is through diurnal stomatal conductance measurements (McDowell et al., 2008). However, carbon isotope analysis can also be used as evidence to which leaf hydraulic strategy a plant may use to tolerate water-stressed conditions (Stewart et al., 2005). Analysis of $\delta^{13}C$ as indicator of water-use efficiency (WUE), where higher $\delta^{13}C$ values indicate higher WUE, renders an integrated measure of carbon assimilation per molecule of water lost (Farquahar et al., 1982). Plants that show consistently higher (less negative $\delta^{13}C$) WUE indicate closure of stomata during drought-stressed times. Studies of $\delta^{13}C$ have provided strong evidence that some species are indeed more WUE than others, especially across different water availability gradients (Stewart et al., 1995; Ares and Fownes., 1999; Ahkter et al., 2005; De Souza et al., 2005). It has also been suggested that rapid alien invasion, specifically in fynbos, can be partially attributed to physiological factors such as plant WUE (Kraaij and Cramer, 1999).

Therefore, the importance of ensuring safe water transport during the soil-plant-atmosphere continuum is cardinal to the survival strategy of different species, and thus a determinant of community structure, as any obstruction in the hydraulic structure of the plant may cause it to experience a loss of turgor at the leaf level. Thus safe and efficient water transport is crucial to maintain uptake of carbon in exchange for water at the mesophyll-atmosphere interface in the leaves. When water-stress is experienced, stomata close, and this could be measured directly, or by proxies such as carbon isotopic profiles of leaf tissues. In

extreme conditions a lack of efficient and safe transport from the root to the leaf may result in individuals for some species wilting and dying, while others are less prone.

1.5.2. Cavitation as a Threat to Plant Hydraulic Systems

An important threat that plant hydraulic systems face is xylem cavitation (Sperry, 1995). This is the phenomenon where air seeds into a vessel (angiosperms) or a tracheid (conifers) under environmental stress, causing an embolus to form in the conduit and thus leading to a substantial loss in the hydraulic conductance of the plant, resulting in either dieback or a reduction in growth (Sperry and Tyree, 1988). Sperry (1995) also described two main environmental drivers that cause cavitation through embolism: freeze-thaw events and water-stress (drought induced). As freezing-induced cavitation is not relevant to the south-western Cape area in South Africa (except maybe for a small part at higher altitudes), I will only discuss drought-induced cavitation further.

The mechanism of water-stress induced cavitation relies on what is now widely known and accepted as the 'air-seeding' hypothesis (as reviewed in Hacke and Sperry, 2001). According to this hypothesis, air will enter a water filled conduit when the pressure difference between the xylem water and the air from the empty adjacent vessel exceeds the capillary forces at the air-water interface, illustrated in Figure 1.1 (Hacke and Sperry, 2001). So, for embolism to occur, the adjacent conduit with which a pit pair is formed has to be already embolized (Hargrave et al., 1994). Air thus enters through inter-conduit pit membrane. Air seeding will usually occur through the largest of these pit pores (Sperry and Tyree, 1988; Pockman et al., 1995). A recent study showed that there exists a positive relationship between the pit area per vessel and vulnerability to cavitation (Wheeler et al., 2005). When the pit membrane area gets smaller, the chances are that the larger more porous apertures will be less abundant and thus that the plant may be less prone to cavitation (Christman et al., 2009). It is clear that there is a safety trade-off between resisting cavitation and having more porous pit membranes which would assist better hydraulic conductance and thus growth (Wheeler et al., 2005). This trade-off will be discussed below.

1.5.3. Measuring Embolism and its effect on Xylem Hydraulic Conductivity

Modelling the expected impact of global climate change on European plant diversity Thuiller et al. (2005) showed that by 2080 more than half of the species will be either vulnerable or threatened. Their study also showed that the drivers for this increased risk in

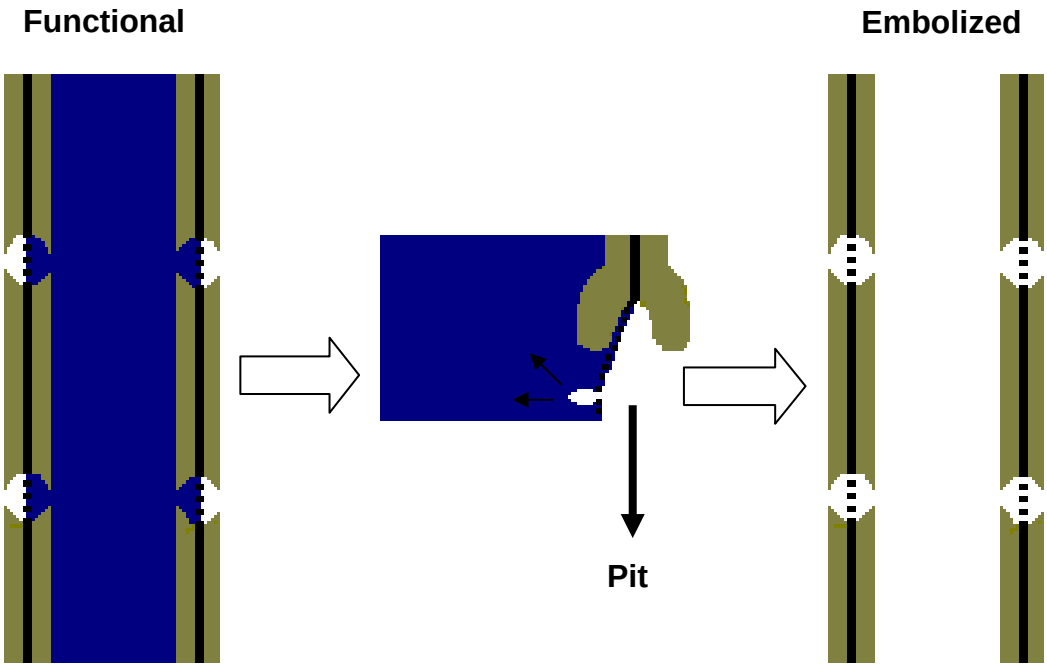

Figure 1.1. Drought-induced cavitation is caused by air entering the functional conduit through the inter-conduit pit membrane due to high pressures in the functional conduit that draw air from adjacent already embolized conduits (adapted from Hacke and Sperry, 2001).

biodiversity loss could be attributed mainly to changes in temperature and moisture availability. In a study which developed scenarios illustrating the possible effects of climate change on woody trees in the Mediterranean Basin, Martinez-Vilalta et al. (2002) noted that the survival expectancy of *Quercus ilex* and *Phyllyrea latifolia* decreased as temperature and evaporation increased. Still, *P. latifolia* had the better chance for survival of the two species and this was attributed to it being less vulnerable to drought-induced cavitation (Martinez-Vilalta et al., 2002). In other words, sudden drought conditions induced by climate change may be better handled by *P. latifolia* relative to *Q. ilex*. These findings indicate the importance and relevance of scientists focussing on a more mechanistic way of understanding how the physical environment influences plant hydraulic functioning to help predict the impacts of changing environmental conditions.

Sperry et al. (1988) designed a method of measuring xylem cavitation and more specifically, certain plants' vulnerability to cavitation. This method essentially correlates water potentials against loss in hydraulic conductivity due to chronic water deficit (Tyree and Sperry, 1988). The end result is a vulnerability curve such as that shown in Figure 1.2. It is also useful to compare inter-specific vulnerabilities to cavitation by interpreting the water potentials of species at the point where 50% of xylem vessels are cavitated, and thus 50% hydraulic conductance is lost (Hacke et al., 2000; Wheeler et al., 2005). This concept arose

from the idea that when plants under pressure endure stress and subsequently become embolized, there will be added stress to provide the leaves with water, giving room for more vessels to be affected and so uncontrolled cavitation rates will continue (Tyree and Sperry, 1988).

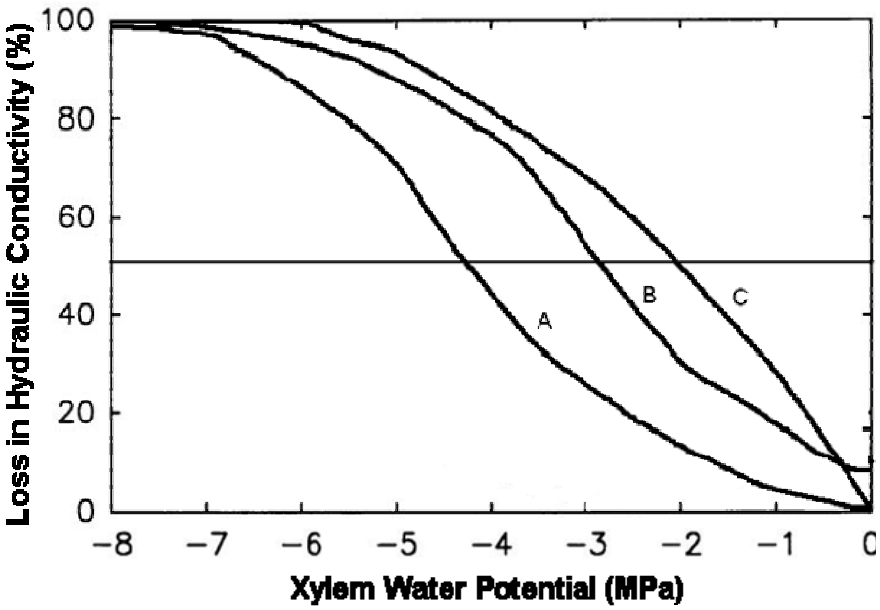

Figure 1.2. Plotting stem water potential against its % loss in conductivity creates a drought vulnerability curve. Measuring the water potential where 50% of the xylem is cavitated (horizontal line) allows a comparison between species of vulnerability to drought. If a plant can survive by having a low water potential (more negative) and still not become subject to cavitation relative to other species, it is more drought-tolerant. Here, species A is more drought-tolerant than the rest (adapted from Tyree and Sperry, 1988)

This phenomenon has been aptly termed "runaway cavitation" (Tyree and Sperry, 1988), which describes its chaotic nature. Runaway cavitation is that amount of cavitation from which the plant will not easily recover and therefore is likely to cause dieback. Runaway cavitation is believed to occur when the plant reaches the P_{50} level (after 50% loss in hydraulic conductivity occurs) (e.g. Hacke et al., 2000; Maherali et al., 2006; Swift et al., 2008). Vulnerability to cavitation measurements thus allows us to compare water potentials of suites of species against their cavitation percentages associated with runaway cavitation. In doing so, logical deductions can be made about their minimum water potentials in a given environment. This has great relevance to ecological range and distribution studies e.g., are plants mesic or xeric, and, what the minimum water potential of a plant species must be for it to remain physiologically active (Bashkar and Ackerly, 2006). All of these measurements can provide vital insights into biogeography and restoration ecology, especially as P_{50} values reflect short-term adaptation to water availability (Maherali et al., 2004).

From Figure 1.2 it is possible to explain why species A is likely to be the most resistant to cavitation and thus the most drought-tolerant, and species C, the least. Species A only reaches P_{50} at around -4.5 MPa, in contrast to C which reaches that same value at around -2 MPa. Comparisons of vulnerability to cavitation among co-occurring species in a community have found that some species reached P_{50} at water potentials that were eight times lower than the most drought vulnerable species, indicating the variety of drought-tolerance strategies that can exist in a single community (Maherali et al., 2006). Studies of this type can define species drought-tolerance and so display a range of water potentials that a species can sustain (Hacke et al., 2000). This method of measuring cavitation has been shown to relate to inter-species physiological differences to xylem water conducting under different water regimes (as reviewed by Pockman et al., 1995).

The study of vulnerability to cavitation has been applied to many different ecological questions. It has shown that variation in drought resistance exists even between ecotypes along a water availability gradient, thus shedding light on intra-species' genetically-based differences and how that helps adaptation to different environmental gradients (Kolb and Sperry, 1999). When Pockman and Sperry (2000) asked questions about the distribution of vegetation in the Sonoran Desert, they found that vulnerability to cavitation, as the underlying mechanism of distribution, can be a valuable tool in illustrating observed plant distributions along a water availability gradient, within a community, or even between broad habitat types. Pockman and Sperry (2000) observed that the upland species had lower water potentials than the obligate riparian species, which makes sense as the latter have more access to water. In addition, the riparian species are less likely to grow in upland areas because cavitation will cause most of their xylem to stop functioning at water potentials as low as those recorded for upland species. In conclusion, mechanistic information of this type is vital for interpreting future distribution and performance of plants, and emphasises the importance of ecophysiological studies as a means to gain a more mechanistic understanding of plant traits that determine their distribution and habitat preference (Pockman and Sperry, 2000).

This study of vulnerability to cavitation also has agricultural applications. Drought sensitive interbreeding willows (*Salix* spp.) that are intensely used for biomass production showed differences in drought adaptation (Wikberg and Ögren, 2004). These findings not only gave insight into the chances of genetically manipulating cultivars to be more drought resistant, but also to incorporate the possible tradeoffs when doing so, such as gaining higher wood density, which improves cavitation-resistance, but leads to lower growth height. Effects of xylem cavitation on agricultural fruit trees such as *Prunus* species have also rendered vital information (Cochard et al., 2008). This economically important genus

contains fruits like peaches and apricots, and should climatic changes render these species less adapted, there is likely to be substantial financial and thus socio-economical losses. Cochard et al. (2008) revealed significant variation in drought adaptation between the species in the genus. These initial results may inspire further studies that can help with refining the most drought vulnerable species in water limited scenarios. In summary, screening vulnerability to drought at this level could be very important for future economic wellbeing.

Directly relevant to Mediterranean-type riparian ecosystems is a recent study done in the Cape Floristic Region fynbos riparian zone (Swift et al., 2008). Vulnerability of four key native species was compared across varying streamflow regimes as a proxy for water availability. This study was carried out within one catchment on the Eerste River and several of its tributaries. Findings suggested that even if these species occur in the riparian zone, and thus have ample water supply, a reduction in that water supply may affect the vulnerability to cavitation of some species substantially. Significant variation was found in the ability of species to withstand cavitation in areas from high to intermediate to low water flow. This is in contrast to the observation that not all riparian species utilize water directly from the streamside, but rather from the water available in the substrata itself by being deep-rooted (Dawson and Ehleringer, 1991). Therefore, further investigation of the hypothesis that flow volume has a marked effect on the drought-tolerance of species as set out by Swift et al. (2008) would provide great additional insights on a) riparian ecology and b) optimising assessment protocols of river sites to prioritise areas for alien eradication and riparian restoration. The implication of these types of studies is knowledge for planning restoration in disturbed riparian zones, such as those associated with clearing post-invasion, since vulnerability to cavitation studies allow comparison between native and invasive species as well as providing a mechanistic understanding as to which species have competitive advantage in a given environment (e.g. Pratt and Black, 2006).

A question remains – if cavitation-resistance is such an important component of drought-tolerance, why is it that this trait has not been selected for in all species? (Maherali et al., 2004). Literature suggests that trade-offs occur between wood density and hydraulic conductance (Hacke and Sperry, 2001; Hacke et al., 2001; Meinzer 2003).

1.5.4. Wood Density and Hydraulic Conductance: Safety vs. Efficiency

Wood density is known to be an important role-player in plant water transport (Meinzer, 2003), and is also responsive to environmental factors such as water availability and temperature (Hacke et al 2001; Thomas et al., 2007). Woody species that allocate more

wood tissue to their stems, and thus become more dense, have been shown to have a slower growth rate compared to trees that consist of less dense wood (Enquist et al., 1999). Although denser wood could increase tree life span (Enquist et al., 1999), it could also decrease hydraulic conductivity of the stems (Wagner et al., 1998; Stratton et al., 2000). This is attributed to xylem conduit diameter and the Hagen-Poiseuille Law (Tyree and Zimmerman, 2002) which states that an increase in vessel diameter would increase stem hydraulic conductivity, yet also result in less dense wood as the percentage of lumen area per transverse section of xylem is increased, thus limiting mechanical strength (Wagner et al., 1998). Wood with smaller conduit diameters thus has reduced hydraulic conductivity (Preston et al., 2006). The Hagen-Poiseuille Law confirms these findings. It implies that vessel lumen conductivity will increase with the fourth power of lumen diameter (Tyree and Zimmerman, 2002; Sperry et al., 2006). For example, should the radius of one vessel only be 90% of the radius of another, then according to Poiseuille's Formula $(0.9r)^4 = 0.66r^4$ - thus, flow rate in the vessel will drop by 34% due to a mere 10% drop in radius (Butlin and Brimicombe, 1998). This variation in vessel diameter and density adds to our understanding of plant strategies in drier areas, and explains how plants balance water availability and leaf evaporative demand (Shume et al., 2004).

Wood density thus affects hydraulic conductance and so describes water transport efficiency, but what ecological safety trade-off exists when selecting for improved or restrained hydraulic conductance? It has been shown how water potentials at 50% embolism (P_{50}) were positively correlated with larger vessel sizes (Kolb and Sperry, 1999). Therefore, in areas where species have higher water potentials, vessels tend to have larger diameters and thus improve water movement efficiency through the xylem. So, according to vulnerability curves, the less dense wood will be more prone to cavitation. Other findings confirm that high wood density is an indicator of resistance to cavitation (Hacke et al., 2000; Hacke et al., 2001; Pratt et al., 2007). Another valuable correlation with wood density, in addition to that of P_{50}, is the minimum water potential or midday water potential. Minimum water potentials are good indicators of different plants' access to soil water (Ackerly 2004; Bashkar and Ackerly, 2006). Therefore if density values are correlated against the water potential where the plant is most stressed, one may find that plants that experience the highest amount of stress, due to very low water potentials, are likely to have denser wood (Ackerly, 2004; Jacobsen et al., 2006). This may be due to a tight evolutionary correlation between drought stress and xylem resistance to embolism (Bashkar et al., 2007). Not only are measurements like density and minimum water potentials relatively easy to obtain (Jacobsen et al., 2006), but also, when applied to the South African fynbos, which is predicted to undergo influential

climate change, they could provide vital information on different drought-tolerances of plants and thus aid in assessing species in riparian ecosystems to prioritize restoration initiatives.

1.5.5. Phenotypic Plasticity in Xylem Density – Effect on Species Geographic Distribution

Phenotypic plasticity can be defined as the "environmentally sensitive production of alternative phenotypes by given genotypes" (Hulme, 2008). Of interest is how variable wood anatomy is for native and invasive species across a water availability gradient. A lack of variation (non-plastic) would suggest that species might endure significant stress under reduced water availability. This can provide important insights into current and future geographic distributions of species – especially that of IAP (Hulme, 2008). Plasticity in wood density measurements also allows us to determine whether plants have the ability to functionally converge in traits that favour the environmental gradient they occur in (Meinzer, 2003). With the literature backing up the importance of density in hydraulic traits and thus also distribution dynamics, it would be instructive to see how one species can vary in adaptive strategy given a single variable – water availability.

1.5.6. Density, Lumen to Wall Ratios and Cavitation

In angiosperms one cannot correlate conduit diameter *per se* against water-stress vulnerability as there was found to be a weak relationship (Hacke and Sperry, 2001). This is because water-stress vulnerability is influenced by inter-conduit pit membranes. When measuring drought-induced cavitation, it seems that conduit diameter might be too plastic to obtain significant correlations (Gorsuch et al., 2001). In *freezing-induced* cavitation events however, conduit diameters do influence vulnerability to cavitation (Davis et al., 1999). In contrast, it has been observed that density correlates significantly and positively with the square of the wall to lumen ratios $(t/b)^2$ (Hacke et al., 2001; Hacke and Sperry, 2001). Because of the negative pressures in the vessels, there exists some strain or bending pressures between two adjacent conduits (Hacke et al., 2001). Therefore it is suggested that if an embolized vessel occurs adjacent to a healthy one, and the wall thickness between them is not sufficiently strong, it might lead to implosion of the conduit. Although implosion is rarely seen, the effect of that bending stress can assist air entry through the pits (Hacke et al., 2001). Figure 1.3 illustrates this phenomenon (Hacke et al., 2001). $(t/b)^2$ also correlates positively with P_{50} and is said to be a valuable screening experiment for comparing drought-tolerance (Cochard et al., 2008).

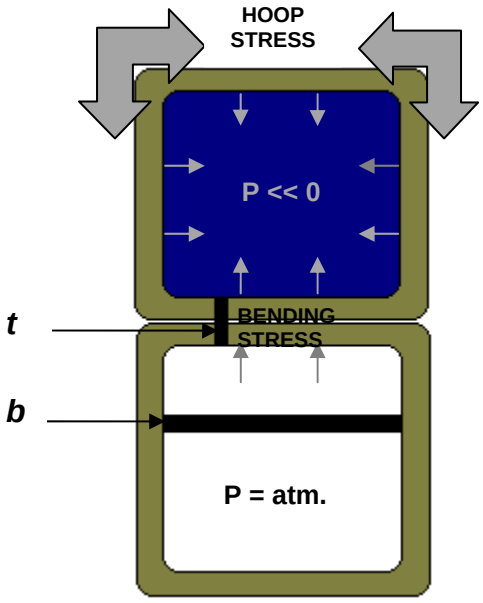

Figure 1.3. Bending and hoop stress due to negative water pressures can result in wall bending (shaded block represents water filled). This bending stress may cause walls to implode and subsequently let air in as shown in figure 1.1 (adapted from Hacke et al., 2001).

Research indicates that a greater degree of mechanical strength is positively and significantly correlated with greater cavitation-resistance (Pratt et al., 2007); and so plants that grow in places with lower water potentials require stronger wall thickness to resist implosion and cavitation (Sperry et al., 2006). Therefore wood density is likely to be closely related to soil water content since, the drier the soil, the denser the wood, and the lower the minimum water potential (Preston et al., 2006).

1.6. Why is this Knowledge Important?

Improved conservation of riparian zones in South Africa is imperative to sustain crucial ecosystem services such as quality and quantity of freshwater. In a changing environment, uncertainty when prioritizing areas for conservation and a lack of cost-efficiency when implementing these schemes may prove detrimental to the future of the natural, social and economic sectors. My review indicates the need to understand and predict IAP distributions in a changing environment, especially under future drought projections, as amplified land-use, global climate change and increased plant invasion continue to work in synergy to reduce an already limited resource. In addition, information is needed to optimise

restoration programmes in terms of identifying areas that need preference in clearing and indentifying plant species that are best suited to the conditions in those areas.

Effectively there is a need for a more mechanistic approach to restoration ecology, i.e. to understand the physical forces that drive the function and ecology of a species in the riparian zone (Esler et al., 2008). Prins et al. (2004) and Galatowitsch and Richardson, (2005) found *Brabejum stellatifolium* and *Metrosideros angustifolia* to be key species in fynbos riparian ecotones, and suggested that restoration efforts should focus on these species. In an attempt to address this issue Swift et al. (2008) investigated specifically the physiology of *Brabejum stellatifolium, Metrosideros angustifolia*, as well as two other important woody species, *Rapanea melanophloeos* and *Brachyleana neriifolia*, both also prominent in riparian zones. By using the vulnerability to cavitation technique to distinguish plant-specific response to streamflow (used as surrogate of plant water availability in near-stream environments), they were able to show that *Brabejum stellatifolium* was highly adaptable over the different areas, and showed a good relationship with streamflow. There is some disagreement in terms of the use of streamflow as a surrogate as some streamside trees may not be tapping into surface water (e.g. Dawson and Ehleringer 1991). It will nevertheless prove insightful to test their hypothesis that streamflow indicates plant water availability over a range of riparian environments and using the same species. However, given that alien invasive species, especially *Acacia* spp. are a major threat to the functioning of riparian ecotones, this need to be extended to also include alien species.

More research in the field of restoration ecology may therefore prove vital in gaining a deeper understanding of not only plant functional traits and life history strategies, but also of the requirements for effective riparian restoration, specifically pertaining to improving cost-to-benefit estimations of alien clearing programmes such as Working for Water. For example, evidence indicated that improved recovery of alien cleared riparian zones in the fynbos biome may rely on re-planting of key indigenous species such as *Brabejum stellatifolium, Metrosideros angustifolia, Erica caffra* (Galatowitsch and Richardson, 2005), *Kiggelaria africana*, *Olea europaea* subsp. *africana* and *Salix mucronata* subsp. *mucronata* (Meek et al., 2009). Therefore, research on ecophysiological and wood anatomical traits on these common species could provide valuable information as to the best candidates for re-vegetation under current climate change models. This knowledge will also contribute to other gaps in literature for example riparian woody species regeneration (Holmes et al., 2005). By defining key tolerant species, one can encourage additional studies to focus on that species' seedling regeneration and thus contribute further to the effectiveness of a restoration management plan.

1.7. Problem Statement

Woody plants display different physiological and wood anatomical hydraulic traits over a given water availability gradient. Cavitation-resistance plays an important role in determining vegetation distribution along these gradients, and can provide a mechanistic understanding of habitat preference. There is a cost of cavitation-resistance to mesic plants and trade-offs consequently exist between safety against cavitation and hydraulic conductance. There are many testable correlations between the physiological traits (P_{50}), wood tissue traits (wood density) and wood cell traits ($(t/b)^2$). From this ecophysiological and anatomical approach, we can answer many questions related to plant dynamics in a community. Therefore, the over-arching question of this research project was:

How vulnerable are selected native and invasive woody riparian species to drought-induced streamflow variability in the Western Cape fynbos ecosystem?

The primary focus was on south-western Cape riparian systems that form part of the fynbos Mediterranean-type ecosystem. Subsequently tests were done on plant physiology (vulnerability to cavitation) and wood anatomical traits (wood density, lumen to wall ratios, vessel lumen and wall diameters), relating to key questions derived from the over-arching question.

1.8. Key Questions Investigated

Key questions were investigated in two data chapters, structured as follows:

Pertaining to Chapter 2 (Physiological Data):

1. In a water-limited scenario, how do key native fynbos riparian woody tree species compare in vulnerability to drought-induced cavitation, xylem water potentials and WUE (an integrator of leaf-level water regulation) against the woody tree invasive *Acacia mearnsii*?

2. What key native species will be most suited for restoration?

3. Could streamflow be a robust screening tool when predicting which sites would be most vulnerable to cavitation, as one would expect species at high flow sites to be less drought-tolerant, than those in low flow areas (sensu Swift et al., 2008)?

Pertaining to Chapter 3 (Anatomical Data):

4. At a narrow geographic range, how would the invasive *Acacia mearnsii* and two key native riparian tree species respond with regards to wood density across three sites that differ in streamflow (sensu Swift et al., 2008)? What do anatomical characteristics contribute?

5. At a wider geographic range, how plastic is *Acacia mearnsii* across sites with different precipitation, and within sites, how does its wood density compare to that of a co-occurring native species?

Chapters 2 and 3 are written as stand-alone research papers; consequently some necessary degree of repetition may be encountered. Chapter 4 is the general discussion and summarises the key findings of each data chapter, and also integrates chapters 2 and 3 to investigate the link between physiology and anatomy. Key recommendations for riparian management and future research opportunities are also discussed.

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Chapter 2

Drought-tolerance of Selected Alien and Native Tree species in Fynbos Riparian Ecotones

Abstract

The Mediterranean-type riparian zones of the south-western Cape are predicted to endure severe future water shortages. Invasive alien plants, such as Acacia mearnsii*, aggravate this problem, hence the initiation of many eradication programs. Unfortunately, the scientific knowledge to prioritize sites for clearing is often lacking, inhibiting cost effectiveness. Vulnerability to drought-induced cavitation, an important measure of drought-tolerance in trees, provides vital information concerning future species distribution and behaviour in a changing environment. A mechanistic approach was therefore used to investigate how key native fynbos riparian woody tree species compare in vulnerability to drought-induced cavitation against* Acacia mearnsii*, by comparing findings from three Mediterranean-type fynbos river systems that differ in streamflow.* Acacia mearnsii *showed lower* P_{50} *values than both native species – an indication of being more drought-tolerant overall and will most likely persist in future drier conditions, therefore remaining top priority to eradicate.* Brabejum stellatifolium *had consistently higher water potentials across all sites than the other studied species, and is proposed to be a valuable species for restoration of south-western Cape riparian zones. Streamflow was shown to be an inaccurate predictor of species drought-tolerance along these riparian systems. Consistency in the shapes of species vulnerability curves across sites, illustrated a species-specific hydraulic response to different water availability – strengthening the argument that this approach to distinguish site-level drought-tolerance between trees is a practical technique, with great application in understanding future geographic distribution under climate change, and potential for use in restoration research.*

2.1. Introduction

South Africa is a water scarce country, where only 8.6% of average rainfall (450 mm per year) becomes available as surface water, one of the lowest conversion ratios in the world. With South African demographic models indicating elevated population growth, stress on water resources will increase with greater demands by the agricultural (irrigation) and urban development (domestic) sectors (Davies and Day, 1998). As a result, freshwater in South Africa is increasingly widely recognised as a limiting resource that will severely inhibit future socio-economic growth (Walmsley et al., 1999). Global climate change is a reality that could aggravate the already limited water situation (Schulze et al., 2001; Meadows, 2006). Climate change predictions indicate that Mediterranean-type ecosystem rivers in the south-western Cape may lose between 14% and 32% of their total streamflow (New, 2002). These hydrological systems are therefore highly vulnerable and there is justifiable concern for matters pertaining to water resource management. Invasions by alien plant species aggravate this problem.

Invasive alien plants (IAPs) are a global concern as they have an enormous impact on biodiversity, water resources and ecosystem services in South Africa. Many transformer species (species that can change the nature of ecosystems over large areas) in South Africa were first introduced to assist in combating soil erosion, and to provide fuel wood and other wood products (Richardson, 1998). The loss in water resources associated with IAPs in South Africa has especially caused concern. The effects that invasive alien timber plantations and escapees from plantations have on water resources, particularly streamflow quantities has received some attention (Bosch and Hewlett, 1982; Le Maitre et al., 1996; Scott et al., 1998; Le Maitre et al., 2002; Dye and Jarmain, 2004). Results indicate a significant reduction in streamflow quantity. In addition, some IAPs elsewhere are also known to have high resistance to drought-induced xylem cavitation (Maherali et al., 2004), and thus have the potential to outcompete native species and persist under drier conditions predicted for many parts of South Africa. However, it is not known whether this is the case with woody IAPs in South Africa.

Riparian zones support some of the densest stands of woody IAPs (Le Maitre et al., 2002; Dye and Jarmain, 2004). Dye and Jarmain (2004) reported that dense stands of *Acacia mearnsii*, especially in riparian zones, had high transpiration rates, resulting in significant reductions in riverflows. Removal of IAPs from riparian environments also leads to significant increases in streamflow (Scott et al., 1998). Therefore by managing the integrity of the riparian zone through removing invasive woody biomass, river systems may continue

to provide crucial ecosystem services (Tabacchi et al., 1998). Loss of ecosystem services prompted the establishment of the Working for Water programme (Van Wilgen et al., 1998) which aims to control selected IAPs. However, a challenge this programme currently faces is the prioritization of areas for clearing, especially in the face of dwindling river . One uncertainty is whether in some areas native species may be better able to persist due to inherently higher drought-tolerance than the invaders, but scientific evidence for this is lacking. This implies a need for more research into the role of inherent physiological abilities of native and invasive riparian woody species to enable persistence in river systems with different water availabilities (Esler et al., 2008). Investigating which IAPs pose the greatest risk to the native riparian vegetation, and where, can aid strategic planning and expenditure and ultimately more effective conservation management (Richardson and Van Wilgen, 2004). Secondly, more research is needed to optimize the suite of species that are replanted after clearing, so as to prevent costly replanting of species within areas where high rates of mortality will result due to constraints in water supplies (Van Wilgen et al., 1998). A review by Holmes et al. (2005) highlighted the need to re-introduce native trees after IAP clearing to optimize post-clearing maintenance and so to prevent re-invasion. They concluded that the feasibility of such restoration strategies may be obstructed by a lack of evidence that the cost of the restoration will match the benefits. Emphasis is thus on cost efficiency through correct decision making. A study indicating differences in vulnerability of native fynbos riparian vegetation to future reductions in streamflow has shown the value of this type of research (Swift et al., 2008).

In order to understand the physiological basis for plant adaptations to drought, Sperry et al. (1988) developed a technique that measures the level of xylem cavitation. This is the phenomenon where air seeds into a vessel (angiosperms) or a tracheid (conifers), causing an embolus to form in the conduits thus leading to a substantial loss in the hydraulic conductance of the plant, causing either dieback or a reduction in growth (Sperry and Tyree, 1988). Two main environmental drivers cause cavitation through embolism: freeze-thaw events and water-stress (Sperry, 1995). This method of Sperry et al. (1988) essentially relates xylem water potentials to loss in xylem hydraulic conductivity due to chronic water deficit (Tyree and Sperry, 1988). The end result is a vulnerability curve, which is useful to evaluate different species' vulnerability to cavitation by comparing xylem water potential against the point where 50% hydraulic conductivity is lost (50% of xylem cavitated), referred to as the P_{50} value (e.g. Hacke et al., 2000; Wheeler et al., 2005; Maherali et al., 2006; Swift et al., 2008). It is seen as the most important parameter in determining drought-tolerance in a tree (Cruziat et al., 2002).

The study of vulnerability to cavitation has been applied to many different ecological questions, e.g. determining the variation in drought-tolerance between subspecies (Kolb and Sperry, 1999); determining the underlying mechanism of tree distribution (Pockman and Sperry, 2000); variance in drought resistance between important agricultural cultivars (Wikberg and Ögren, 2004; Cochard et al., 2008); and possible impacts of climate change on species survival (Martinez-Vilalta et al., 2002). It has also been applied to restoration ecology (Swift et al., 2008). Swift et al. (2008) showed how key riparian species in the South African fynbos region had differences in their vulnerability to drought-induced cavitation, with the least vulnerable species observed at low streamflow sites. This implies that streamflow may be a valuable proxy for indicating plant water availability although this is not always the correct assumption for riparian species (Dawson and Ehleringer, 1991). Findings of Swift et al. (2008) proved insightful pertaining to selecting species for restoration and site prioritisation. Further exploration of the hypothesis put forward by Swift et al. (2008) that streamflow volume is strongly linked to drought-tolerance could provide valuable information for restoration managers as a robust tool to predict sites where plants might experience drought-stress more intensively. All of this is vital information concerning future species distribution and behaviour, emphasising the importance and relevance of focusing on a more mechanistic way of understanding environmental effects on plant hydraulic functioning. Results of these studies are thus also central to planning restoration in disturbed riparian zones, since this allows comparison between native and invasive species – to see which species might have competitive advantage in a given environment (e.g. Pratt and Black, 2006).

Drought-tolerance must be seen in conjunction with leaf level regulation of water as different plants may adopt different ecological strategies to cope under dry conditions (Lambers et al.,1998). A recent framework by McDowell et al. (2008) emphasized the two main strategies that plants may use to cope with declining water availability. On the one hand, some plants may continue carbon assimilation by keeping stomata open under low water potentials (anisohydry). This is a risky strategy under short intense droughts as water potentials might exceed the plants' safety threshold (i.e. drop below P_{50} tensions), and this may cause dieback of branches, or kill the whole plant. On the other hand, species close their stomata to prevent excessive loss of water under dry conditions, in so doing relieving cavitation pressure on plants (isohydry). This strategy is also risky under extended drought conditions, which would lead to carbon starvation of the plant. For example, in the study by Swift et al. (2008), key native riparian species *Brabejum stellatifolium* and *Metrosideros angustifolia* seem to differ in their ecological strategies under drier conditions. The minimum

water potentials of *Metrosideros angustifolia* seem to exceed the P_{50} values found, which may suggest that runaway cavitation may result if stomata do not close, with mortality a possibility. On the other hand, *Brabejum stellatifolium* may be able to keep its stomata open under dry conditions as its safety margins are large, with lower probability of exceeding the P_{50} values (Swift et al., 2008). These strategies may manifest in the measured $\delta^{13}C$ values as indicator of water-use efficiency.

Therefore, at the leaf level, I investigated $\delta^{13}C$ as indicator of water-use efficiency (WUE) where higher $\delta^{13}C$ values indicate higher WUE (Farquahar et al., 1982). $\delta^{13}C$ provides strong evidence that some species are more WUE than others, especially across different water availability gradients (Stewart et al., 1995; Ares and Fownes., 1999; Ahkter et al., 2005; De Souza et al., 2005). Seibt et al. (2008) described the potential of $\delta^{13}C$ in understanding possible effects of global warming on WUE, and subsequent species survival strategies. It has also been suggested that rapid alien invasion, specifically in fynbos, could be attributed to factors such as plant WUE (Kraaij and Cramer, 1999). Therefore knowledge whether invasive species outcompete native species at the whole plant level (less vulnerable to cavitation) and the leaf level (better WUE) could prove highly insightful.

This study therefore addressed the following key questions: 1) In a water-limited scenario, how do key native fynbos riparian woody tree species compare in vulnerability to drought induced cavitation, xylem water potentials and WUE (an integrator of leaf-level water regulation) against the woody tree invasive *Acacia mearnsii*? 2) What key native species will be most suited for restoration? 3) Could streamflow be a robust screening tool when predicting which sites would be most vulnerable to cavitation, as one would expect species at high flow sites to be less drought-tolerant, than those in low flow areas (sensu Swift et al., 2008)? I investigated these questions by comparing findings from three Mediterranean-type fynbos river systems that differ in streamflow, ranging from low to high flow.

2.2. Methods

2.2.1. Description of Species and Study Sites

The predominantly riparian native species *Brabejum stellatifolium* (Proteaceae) and *Metrosideros angustifolia* (Myrtaceae) (Coates-Palgrave 2002; Reinecke and King 2007) were selected to study, as these evergreen species, which are the only indigenous species of their genus in South Africa, are key species in south-western Cape Mediterranean-type

riparian systems (Galatowitsch and Richardson, 2005). The physiology of the native species was contrasted with the evergreen invasive woody species *Acacia mearnsii* (Fabaceae), which is a major threat to functioning of riparian zones in the south-western Cape (Dye and Jarmain, 2004). However, *Acacia mearnsii* can also grow in upland areas under low water availability as it is not an obligate riparian species (for example, it is a forestry tree in some parts of South Africa). The question then is, can one really compare riparian species with a species that can inhabit riparian and upland habitats? Research have shown that intra-specific variation in drought-tolerance across wet and dry habitats does exist, where one species that showed high drought-tolerance in the dry areas did not show the same degree of drought-tolerance in wet areas (Mencuccini and Comstock, 1997; Sparks and Black, 1999). We cannot therefore assume that a species will always outcompete the co-occurring riparian species in terms of drought-tolerance just because it can also grow in dryland areas (Pratt and Black, 2006), as functional convergence in traits has been observed in several ecosystems (Meinzer, 2003).

The study sites were the Eerste River at Jonkershoek (33º57.621'S, 18º55.037'E; 200 m a.s.l.), Wit River at Bainskloof (33º34.217'S, 19º08.452'E; 279 m a.s.l.) and Molenaars River at DuToitskloof (33 41.778’S, 19º13.263'E; 297 m a.s.l.) (Figure A2, Appendix A). Site selection was based on the following criteria: the presence of the selected suite of two native and one invasive species; the river system is invaded and no restoration/clearing has occurred and sites differ in streamflow quantity – i.e. ranging from high to low flow. Jonkershoek (JNK) represents the low flow site, Bainskloof (BSK) the intermediate flow site and DuToitskloof (DTK) the high flow site (Figure 2.1).

There are some complicating factors as far as streamflow data are concerned. The streamflow data shown for each of the rivers may be slightly biased by distance of the sampling sites to the location of the weirs, by the occurrence of woody alien species (*Acacia, Populus*, *Quercus, Eucalyptus* and *Pinus*) within the riparian zone, as well as other site specific factors. The reach where the study site in Jonkershoek is located is below the Kleinplaas Dam, operational since 1981, and which regulates water flow close to the natural flow regimes. In addition, the catchment has been afforested since the 1920s (Bosch and Hewlett 1982). This implies that current streamflow rates are in all likelihood significantly lower than what would have been found under prevailing natural conditions prior to 1900. Our sampling site at Jonkershoek was 2.4 km below the weir at Swartbrug, and the weir at Bosboukloof was 0.68 km away from the confluence with the Eerste River (data from both weirs combined to represend the streamflows of the Eerste River). It is conceivable that the streamflow measured may underestimate or overestimate streamflow depending on the relative contribution of shading of the stream, groundwater recharge and other sources of

water to the stream. However, as Jonkershoek is the low flow site, this does not impact significantly on the choice and ranking of sites in terms of streamflow, as both DuToitskloof and Bainskloof sites had notably higher streamflows (Figure 2.1). At both the latter sites, the sampling locations were also closer to the location of the weir (0.54 km upstream from the weir at Bainskloof and 5.7 km downstream from the weir at DuToitskloof).

In Table 2.1 I summarise the river geomorhological characteristics. Jonkershoek and Bainskloof are draining catchments with predominantly sandstone geology, while at DuToitskloof sandstone is mixed with igneous rocks. The Bainskloof site is situated in the Mountain Stream Transitional zone, while the two other sites are in the Upper Foothills, which has a lower gradient. The sampled trees also occupy different elevations above and distances away from the active channel (during low flow conditions). In addition, visually, the highest cover of alien species (including *Acacia*, *Populus* and *Quercus* spp.) was found at Jonkershoek and at DuToitskloof (predominantly *Acacia*), though a recent fire at the latter site considerably reduced the green biomass. At Bainskloof some clearing occurred on the northern Bank, and none on the southern Bank (Reinecke and King, 2007). Our sample trees were located on both banks. No clearing occurred at the particular location the sampling site is located at Bainskloof (Michael 't Sas-Rolfes, personal communication), and similarly at the Jonkershoek site.

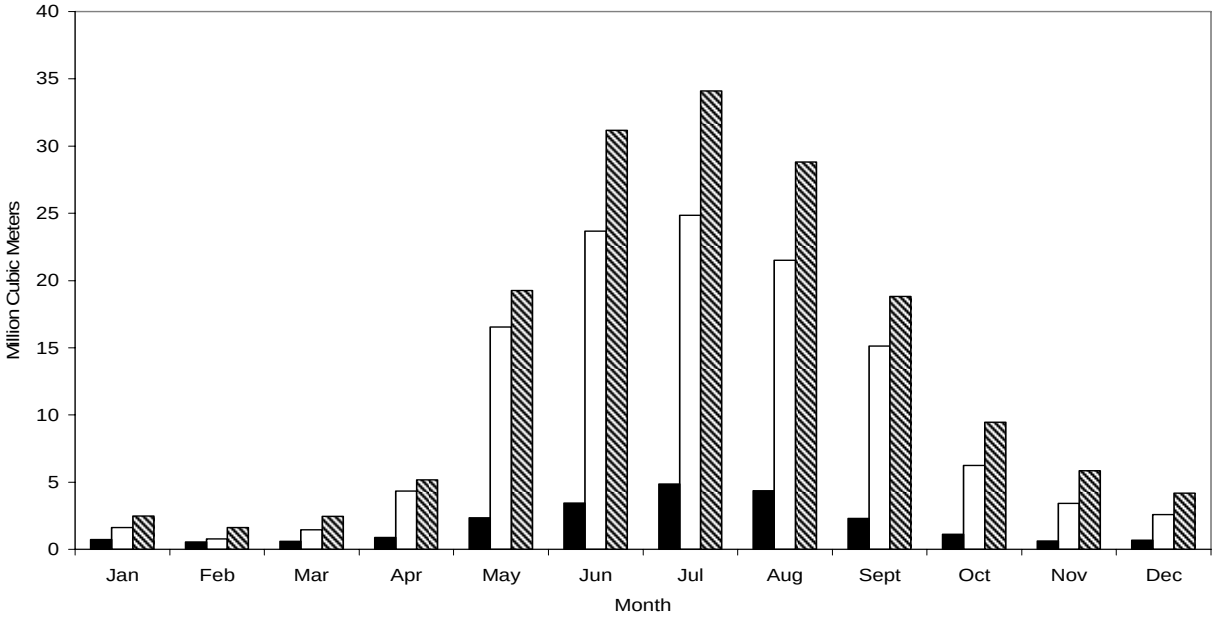

Figure 2.1. Mean monthly streamflow at three study sites representing low, intermediate and high streamflow regimes respectively (from 1970 to 2008). Jonkershoek (black bars), Bainskloof (open bars) and DuToitskloof (cross-hatched bars). Data for Jonkershoek were obtained from the CSIR, and that of DuToitskloof and Bainskloof from Department of Water Affairs and Forestry (now Department of Water Affairs).

Table 2.1. Site-specific information on each of the sites, including the major features of the rivers and the specific river reaches where the sites are situated, as well as pertinent information on the locality of the sample trees in relation to the stream

	Jonkershoek	**Bainskloof**	**DuToitskloof**
River	Eerste	Wit	Molenaars
River type (Naiman and Decamps, 1997)	Perennial	Perennial	Perennial
Geology	Sandstone	Sandstone	Sandstone and Igneous
Characteristic channel features (according to King and Shael, 2001)	Moderately steep, cobble-bed and mixed bedrock-cobble bed channel.	Moderately steep, dominated by bedrock or boulder	Moderately steep, cobble-bed and mixed bedrock-cobble bed channel
Longitudinal Zone (according to King and Shael, 2001)	Upper Foothills	Mountain Stream Transitional Zone	Upper Foothills
Range in the distance of sample trees from streams edge during low flow conditions	<2m	2-10m	<5
Zonation pattern where sample trees were located (Boucher and Tlale, 1999; Sieben and Reinecke, 2008)	Dry bank	Dry bank	Dry bank
Elevation above stream during low flow conditions	<1	1-3m	<1

2.2.2. Vulnerability to Cavitation

Vulnerability to cavitation was measured using the dehydration method (bench drying method) (Sperry et al., 1988; Pockman and Sperry, 2000, Swift et al., 2008). By determining xylem hydraulic conductivity loss due to cavitation, plotted against xylem water potential, drought vulnerability curves for multiple species were obtained (Tyree and Sperry, 1988). Sampling was conducted during the driest months to capture the period of highest drought stress (lowest water potential) (December 2008 – February 2009). Five individuals per species were sampled. Seven branches from each individual were cut at predawn where the first sample was also bagged (to equilibrate transpiration through the plant) for the 0h measurement. The remaining branches were then transported to the laboratory and allowed to dehydrate for 1, 2, 4, 6, 8 and 10h on an open laboratory bench. Before measuring stem hydraulic conductivity at each time interval, branches were bagged and left to equilibrate for 1.5 – 2 hours. Ultimately, dehydration of branches should result in the 10h measurement revealing a 90-100% loss in hydraulic conductivity (xylem 90-100% cavitated). If the required amount of dehydration did not occur, supplementary branches were cut and left for 12-24hs to achieve the desired loss. From each sampled branch, a side branch was cut to measure water potential, using a pressure chamber (Pockman et al., 1995) (PMS instruments, Model 1000, Oregon, USA). The sampled branch was then submerged under water (preventing introduced cavitation) and a stem segment of +/- 10cm was cut and edges trimmed with a sharp blade. Stems were then connected to the tubing apparatus (as described in Sperry et al., 1988) and initial hydraulic conductivity (K_h) was measured. To obtain maximum conductivity (K_{max}), stems were then flushed for 30 minutes with 0.1 micrometer filtered, degassed, de-ionised water, at 0.1 MPa (again to prevent introduced cavitation or blockage) and remeasured. Percentage loss in hydraulic conductivity (PLC) for each stem was calculated as:

$$PLC = 100 \times (1 - K_h/K_{max}) \quad \text{(Equation 1)}$$

Vulnerability curves for each species were fitted with an exponential sigmoidal equation (Pammenter and Vander Willigen, 1998):

$$PLC = 100/ (1 + \exp(a(\Psi - b))), \quad \text{(Equation 2)}$$

where Ψ is the water potential, PLC is the corresponding loss in hydraulic conductivity and *a* and *b* are constants. Water potential where 50% hydraulic conductivity is lost (P_{50}) corresponded to coefficient *b* in equation 2. Curve slope, as an indication of pit membrane size variability, corresponds to coefficient *a* in equation 2. Vulnerability curve slopes have biological relevance in accordance with the 'air-seeding' hypothesis, as there exists an inverse relationship between the pit area per vessel and vulnerability to cavitation (Wheeler et al., 2005). Air seeding will usually occur through the largest pit pores (Sperry and Tyree, 1988; Pockman et al., 1995). Therefore, as pit membrane area declines in size, larger more porous apertures are generally less abundant, leaving the plant less prone to cavitation. Essentially, cavitation at higher water potentials opposed to cavitation at lower water potentials implies the presence of that rare larger pit membrane pore, known to be the underlying cause of cavitation (Choat and Pitterman, 2009; Christman et al., 2009). Thus according to the equation of Pammenter and Vander Willigen (1998), the species indicating more gradual slopes may have more variable pit membrane sizes and therefore cavitate over a wider range of water potentials. Significant differences in P_{50} and curve slopes, within and between species and sites, were identified using a test based on the t-distribution, where Bonferroni multiple testing corrections were applied (Statistica Release 8, StatSoft Inc.).

2.2.3. Xylem Water Potentials in the Field

Stem xylem water potential was measured using a pressure chamber (Pockman et al., 1995) (PMS instruments, Model 1000, Oregon, USA). Measurements were made in summer (December-February), when plants experience drought stress. Predawn (05h00 – 05h30) and midday (12h00-14h00) measurements were made to obtain maximum (predawn) and minimum (midday) water potentials, and were carried out on the same individuals as used for vulnerability curves. Three healthy stems (with leaves) from each individual were cut and measured immediately on site. Three replicates of each treatment were made for each species across all sites. Data were analysed using a factorial ANOVA, to determine interactions between sites, species and time of day (predawn or midday), followed by a Fisher LSD post-hoc test (Statistica Release 8, StatSoft Inc.).

2.2.4. Foliar Carbon Isotope Analysis

For the $\delta^{13}C$ analyses, ten mature (fresh) leaves from each individual (same individuals as used for vulnerability curves and water potentials) were stored in paper bags

and dried at 40ºC until a constant weight was achieved. Leaves were then crushed into a fine powder and analysed for N content, C content and $\delta^{13}C$ by combustion in an automated Elemental Analyzer (Carlo-Erba). The carbon isotope ratio was expressed as:

$$\delta^{13}C\ (‰) = ((R_{sample} - R_{standard}) / R_{standard}) \times 1000 \qquad \text{(Equation 3)}$$

where R_{sample} is the $^{13}C/^{12}C$ ratio of the sample, and $R_{standard}$ the PDB standard.

2.3. Results

2.3.1. Vulnerability of Stem Xylem to Cavitation

Within species, the slopes of vulnerability curves remained relatively constant across sites (Figure 2.2), with only the magnitude of P_{50} values differing (Table 2.2). At the initiation of measurements *Metrosideros angustifolia* and *Acacia mearnsii* always had a percentage of their xylem already embolised, and then lost conductivity gradually as water potential declined. In contrast *Brabejum stellatifolium* started off with near zero percent xylem embolised, but at around -2.5 MPa rapidly started to cavitate. P_{50} values for *Brabejum stellatifolium* were the lowest at Bainskloof (more negative; $P < 0.05$). *Metrosideros angustifolia* at Jonkershoek had a significantly higher (less negative) P_{50} than at Bainskloof ($P < 0.05$), but did not differ from DuToitskloof. *Metrosideros angustifolia* at Bainskloof and DuToitskloof did not differ significantly from one another. There were no significant differences in P_{50} values for *Acacia mearnsii* between all sites. *Brabejum stellatifolium* reached 100% loss in conductivity (P_{100}) between -5.5 and -6.5 MPa at Jonkershoek and DuToitskloof, but for Bainskloof P_{100} was achieved at pressures lower than -7 MPa (Figure 2.2). Across all sites, both *Metrosideros angustifolia* and *Acacia mearnsii*, did not reach P_{100} at pressures higher than -7 MPa (Figure 2.2). All species at Bainskloof consistently showed less loss in conductivity at the -7 MPa level. Overall, *Acacia mearnsii* had the lowest absolute P_{50} values (ranging between -3.70 and -4.43 MPa) and *Metrosideros angustifolia* the highest (ranging between -1.42 and -2.59 MPa).

Within individual sites, consistent patterns were evident in the ranking of species' vulnerability to drought induced cavitation (Figure 2.3, Table 2.2). At Jonkershoek, *Metrosideros angustifolia* had a significantly higher P_{50} ($P < 0.05$) than both other species. This is the same for Bainskloof. At DuToitskloof however, *Acacia mearnsii* had a

significantly lower P_{50} value ($P < 0.05$) than both species. Overall, species at Bainskloof showed the lowest absolute P_{50} values (ranging between -2.59 and -4.43 MPa), and species at Jonkershoek the highest (ranging between -1.42 and -3.70 MPa).

Table 2.2. Mean P_{50} values across species and streamflow regime. All values are in MPa. Standard errors are given in parentheses. A test based on the t-distribution was used to compare estimates of the different streamflow treatments. Bonferroni multiple testing corrections were applied. Within and between sites, means with different superscripts differ significantly ($P < 0.05$).

Site	Streamflow	Species	P_{50}
Jonkershoek	Low	*Brabejum stellatifolium*	-2.86^{bc} (0.09)
		Metrosideros angustifolia	-1.42^{d} (0.14)
		Acacia mearnsii	-3.70^{ab} (0.27)
Bainskloof	Intermediate	*Brabejum stellatifolium*	-3.87^{a} (0.15)
		Metrosideros angustifolia	-2.59^{bc} (0.26)
		Acacia mearnsii	-4.43^{a} (0.43)
DuToitskloof	High	*Brabejum stellatifolium*	-2.66^{c} (0.09)
		Metrosideros angustifolia	-2.26^{cd} (0.22)
		Acacia mearnsii	-3.95^{ab} (0.35)

Table 2.3. R^2 values and probability (P) of fitted vulnerability to cavitation curves for species across all sites

Site	Species	Equation	R^2	P
Jonkershoek	*Brabejum stellatifolium*	y=100/(1+exp((1.75375)*(x - (-2.8606))))	0.76	< 0.001
	Metrosideros angustifolia	y=100/(1+exp((.555544)*(x - (-1.4174))))	0.65	< 0.001
	Acacia mearnsii	y=100/(1+exp((.508276)*(x - (-3.7012))))	0.38	< 0.001
Bainskloof	*Brabejum stellatifolium*	y=100/(1+exp((1.23879)*(x - (-3.8726))))	0.76	< 0.001
	Metrosideros angustifolia	y=100/(1+exp((.414685)*(x - (-2.5904))))	0.46	< 0.001
	Acacia mearnsii	y=100/(1+exp((.306903)*(x - (-4.4311))))	0.35	< 0.001
DuToitskloof	*Brabejum stellatifolium*	y=100/(1+exp((1.7069)*(x - (-2.6617))))	0.90	< 0.001
	Metrosideros angustifolia	y=100/(1+exp((.50287)*(x - (-2.256))))	0.53	< 0.001
	Acacia mearnsii	y=100/(1+exp((.360492)*(x - (-3.9484))))	0.38	< 0.001

Table 2.4. Mean values for summer Pre-Dawn (Ψ_{max}) and Mid-Day (Ψ_{min}) water potentials across species and sites (dry season). All values are in MPa. Standard errors are given in parentheses. A factorial ANOVA was used to compare species and sites, with means separated using Fisher's LSD test. Within and between sites, means with different superscripts differ significantly ($P < 0.05$). n = 15.

Time of Day	Species	Jonkershoek	Bainskloof	DuToitskloof
Pre-Dawn	*Brabejum stellatifolium*	-0.27^{a} (0.02)	-0.66^{cde} (0.08)	-0.32^{a} (0.02)
	Metrosideros angustifolia	-0.36^{a} (0.06)	-1.10^{b} (0.11)	-0.43^{ae} (0.05)
	Acacia mearnsii	-0.52^{ad} (0.02)	-1.50^{f} (0.14)	-1.01^{b} (0.09)
Mid-Day	*Brabejum stellatifolium*	-1.91^{a} (0.08)	-1.95^{a} (0.06)	-1.89^{a} (0.07)
	Metrosideros angustifolia	-2.65^{bd} (0.12)	-2.85^{de} (0.10)	-2.52^{b} (0.07)
	Acacia mearnsii	-2.60^{bd} (0.07)	-2.96^{ce} (0.07)	-2.65^{bd} (0.05)

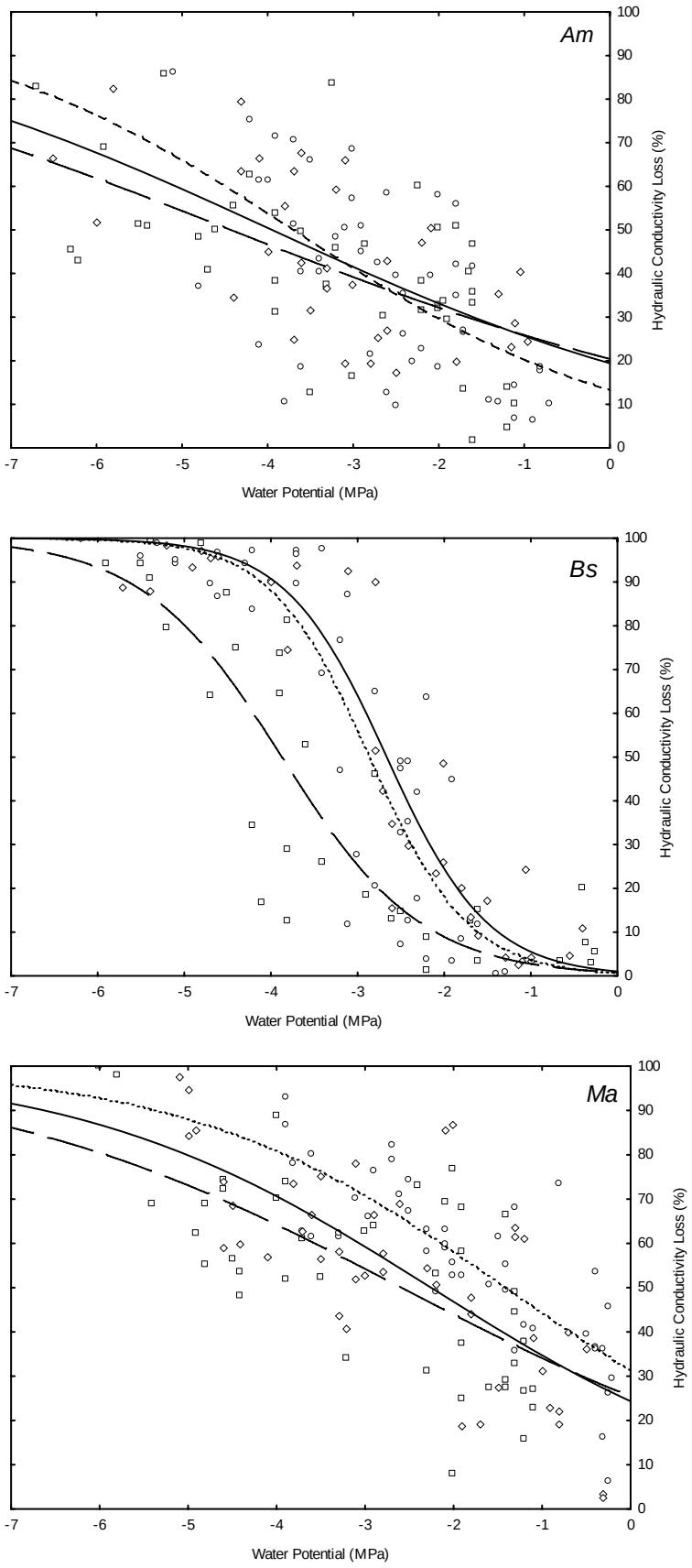

Figure 2.2. Vulnerability of stem xylem to cavitation for *Acacia mearnsii* (*Am*), *Brabejum stellatifolium* (*Bs*) and *Metrosideros angustifolia* (*Ma*). Dotted line (and ○) is Jonkershoek, dashed line (and □) is Bainskloof, and the solid line (and ◊) DuToitskloof. Curves were fitted by an exponential sigmoidal function (Pammenter and Vander Willigen, 1998). Refer to Table 2.3 for R^2 and P values.

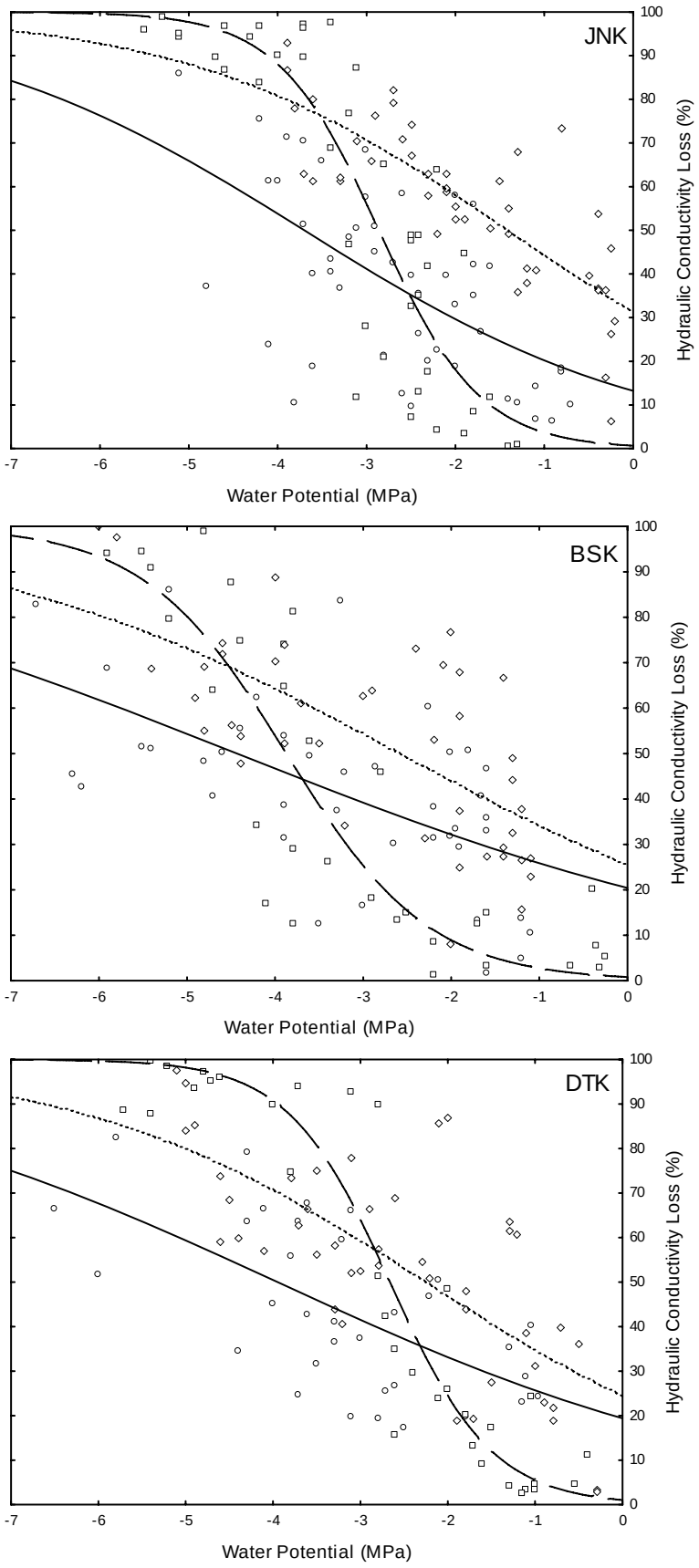

Figure 2.3. Vulnerability of stem xylem to cavitation for the studied species at three sites representing low flow, viz Jonkershoek (JHK), intermediate flow, viz Bainskloof (BSK), and high flow, viz DuToitskloof (DTK), streamflow regimes. At each site, the dotted line (and ◊) is *Metrosideros angustifolia*, dashed line (and □) is *Brabejum stellatifolium*, and the solid line (and ○) *Acacia mearnsii*. Curves were fitted by an exponential sigmoid function (Pammenter and Vander Willigen, 1998). Refer to Table 2.3 for R^2 and P values.

There was a good relationship (R^2) between water potential and percentage loss in conductivity for both *Brabejum stellatifolium* and *Metrosideros angustifolia*, whereas *Acacia mearnsii* showed consistently relatively weak correlations (Table 2.3). However, all of the curves were highly significant.

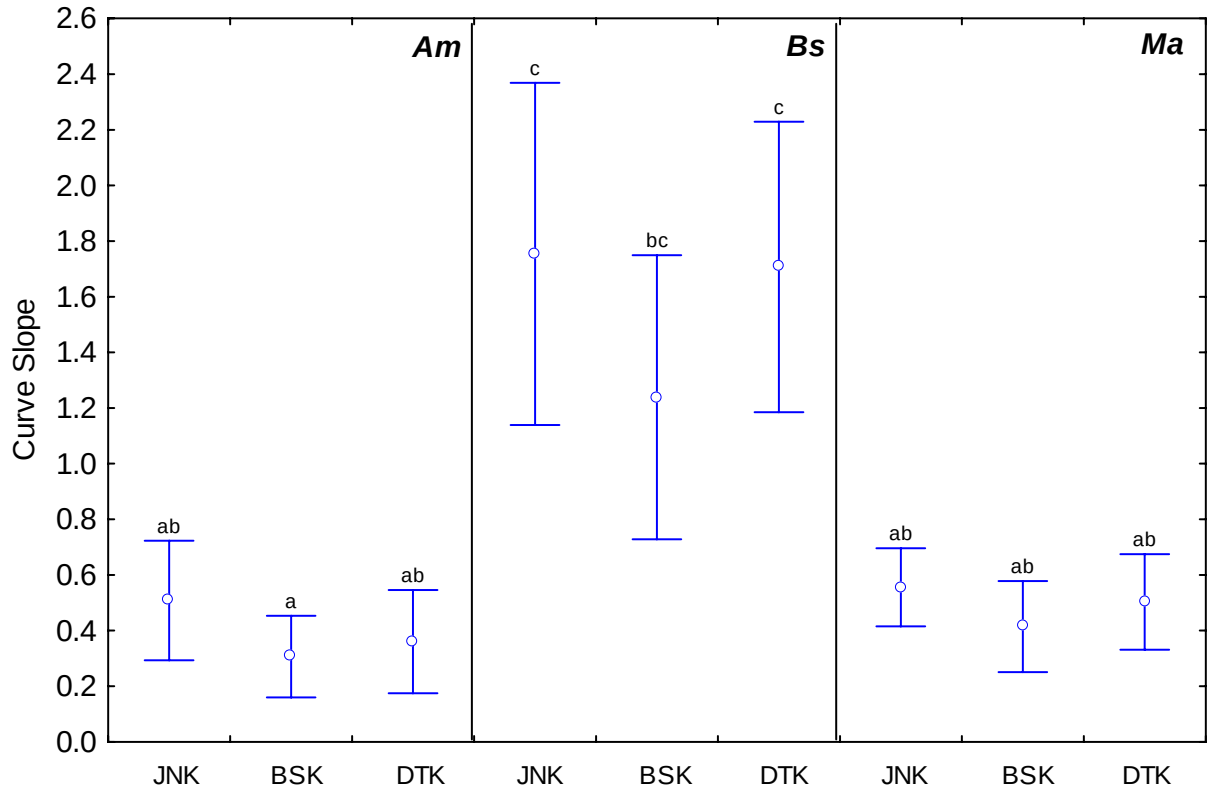

Figure 2.4. Vulnerability curve slopes (coefficient *a* in Pammenter and Vander Willigen (1998)), as a measure of pit membrane size variability in measured stems of *Acacia mearnsii (Am)*, *Brabejum stellatifolium (Bs)* and *Metrosideros angustifolia (Ma)* across three sites, Jonkershoek (JHK), Bainskloof (BSK) and DuToitskloof (DTK). A test based on the t-distribution was used to compare estimates of the different treatments. Bonferroni multiple testing corrections were applied. Within and between sites, means with different superscripts differ significantly ($P < 0.05$).

Slopes of vulnerability curves (as possible indicators of pit membrane size variability in stems) for *Metrosideros angustifolia* and *Acacia mearnsii* across all sites did not differ significantly (Figure 2.4). *Brabejum stellatifolium* however showed an overall significantly higher (steeper) slope than the other species across all sites ($P <0.05$).

2.3.2. Field Xylem Water Potentials

At Bainskloof, all species showed significantly lower pre-dawn water potentials compared to other sites during the measurement period (dry season) ($P <0.05$; Table 2.4). Within *Brabejum stellatifolium* and *Metrosideros angustifolia*, no significant difference

existed in pre-dawn water potentials for Jonkershoek and DuToitskloof. In contrast, pre-dawn water potentials for *Acacia mearnsii* at Jonkershoek were significantly higher than at DuToitskloof ($P < 0.05$). Comparisons between species within individual sites, revealed that species at Jonkershoek showed no significant difference in their predawn water potential. At DuToitskloof, only *Acacia mearnsii* had a significantly lower predawn water potential. At Bainskloof all three species differed significantly in their pre-dawn water potentials ($P < 0.05$), with *Brabejum stellatifolium* having the highest water potential (-0.66 MPa), and *Acacia mearnsii* the lowest (-1.50 MPa).

Within species, across all sites, *Brabejum stellatifolium* showed no significant difference in midday water potential (Table 2.4). *Metrosideros angustifolia* at Jonkershoek did not differ significantly from Bainskloof or DuToitskloof, but had a significantly lower water potential at Bainskloof than at DuToitskloof. For *Acacia mearnsii*, only Bainskloof showed significantly lower midday water potentials ($P < 0.05$). Comparisons between species, within and across sites, revealed that *Brabejum stellatifolium* was consistently less water-stressed at midday than *Metrosideros angustifolia* or *Acacia mearnsii*, which did not differ from one another.

Although significant differences in water potential (both pre-dawn and midday) were found within and between species and sites, there was no interaction between time of day (pre-dawn or midday), species and sites (Figure 2.5).

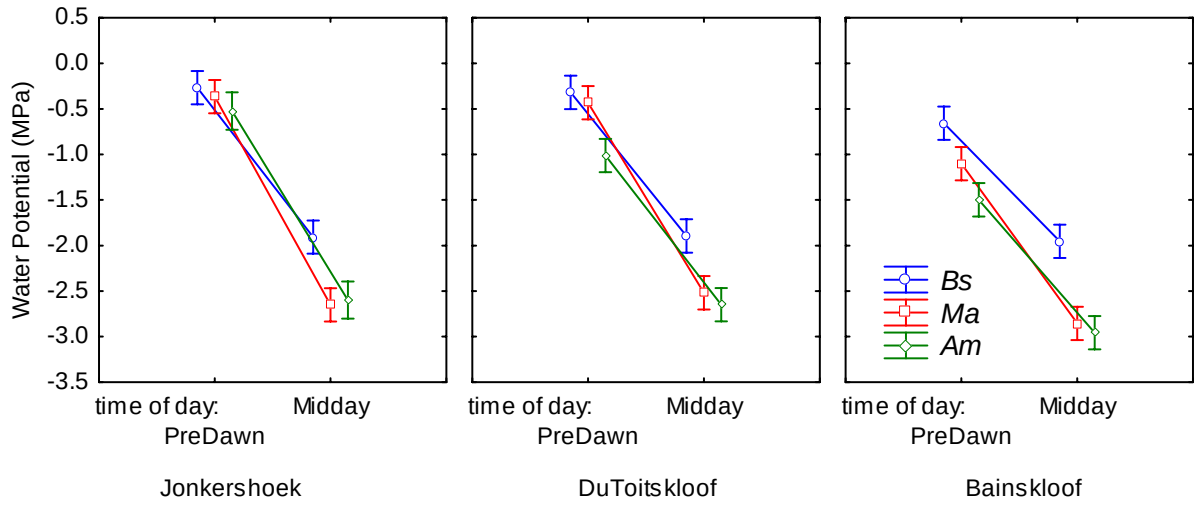

Figure 2.5. Interaction between Pre-Dawn (Ψ_{max}) and Mid-Day (Ψ_{min}) water potentials for *Brabejum stellatifolium (Bs)*, *Metrosideros angustifolia (Ma)* and *Acacia mearnsii (Am)* across all sites. Factorial ANOVA was used to test between sites, species and time of day (pre-dawn or midday), revealing no significant differences (no interaction).

2.3.3. Foliar $\delta^{13}C$

All species showed their lowest $\delta^{13}C$ at DuToitskloof (Table 2.5). *Metrosideros angustifolia* at Jonkershoek and DuToitskloof had a significantly higher $\delta^{13}C$ value ($P < 0.05$) compared to *Acacia mearnsii* and *Brabejum stellatifolium* at these sites. The latter two species showed no significant difference when compared with each another. At Bainskloof, *Brabejum stellatifolium* and *Acacia mearnsii* again did not differ significantly, but here, *Metrosideros angustifolia* had a significantly higher $\delta^{13}C$ value than *Brabejum stellatifolium*, but not *Acacia mearnsii*. Overall, *Metrosideros angustifolia* showed the highest $\delta^{13}C$ values.

Table 2.5. $\delta^{13}C$ as indicator of water use efficiency (WUE). A factorial ANOVA was used to determine species and site interactions. Within sites and species, WUE differ significantly ($P < 0.001$ respectively); there were no significant interactions between sites and species. Means were separated using Fisher's LSD test. Within and between sites, means with different superscripts differ significantly ($P < 0.05$). Standard error is given in parentheses.

Site	Species	$\delta^{13}C$ (‰)
Jonkershoek	*Brabejum stellatifolium*	-25.3^{a} (0.52)
	Metrosideros angustifolia	-23.6^{b} (0.41)
	Acacia mearnsii	-25.4^{a} (0.26)
Bainskloof	*Brabejum stellatifolium*	-24.8^{ad} (0.19)
	Metrosideros angustifolia	-22.7^{b} (0.59)
	Acacia mearnsii	-23.8^{bde} (0.32)
DuToitskloof	*Brabejum stellatifolium*	-26.8^{c} (0.46)
	Metrosideros angustifolia	-25.0^{ae} (0.48)
	Acacia mearnsii	-27.0^{c} (0.41)

2.4. Discussion

It is known that woody plants display species-specific structural and physiological characteristics, as well as species-specific adaptation strategies when subjected to prolonged drought periods (Beikircher and Mayr, 2009). In this study, consistency in the shapes of vulnerability curves of the three selected species across all sites (where only the magnitude of measured P_{50} differed), illustrates that species-specific hydraulic architecture remained relatively intact across sites that differ in water availability. However, the significant changes in magnitude of P_{50} within a species, represented *in situ* responses to different degrees of environmental pressure. Thus, between species, there were diverse responses to hydrological drought (D'Odorico and Porporato, 2006), but within species a consistent trend was observed. Intra-specific differences between populations confirms indications that vulnerability to

cavitation might either be a plastic response to environment (Pockman and Sperry, 2000), or the effect of genetic differences (Kolb and Sperry, 1999). However, more insight and research is needed in this regard (Hacke and Sperry, 2001).

Of the native species studied, *Metrosideros angustifolia* had higher P_{50} values than *Brabejum stellatifolium* at both the low and intermediate flow sites, indicating that the former may be less drought-tolerant (Cochard et al, 2008). However, at the high flow site there was no clear difference in vulnerability to cavitation between the native species. In general, the invasive species showed evidence of being more drought-tolerant across all sites. However, only at DuToitskloof did *Acacia mearnsii* show greater drought-tolerance than both native species, as *Brabejum stellatifolium* did not differ significantly from the invasive species at the other sites. In contrast, *Acacia mearnsii* always showed higher drought-tolerance than *Metrosideros angustifolia*. These results indicate that for the selected native species, *Brabejum stellatifolium* is the most drought-tolerant and thus better adapted in drier conditions than *Metrosideros angustifolia*. This confirms findings from Swift et al. (2008), who worked on the Eerste River and adjacent river catchments in Jonkershoek and surrounds. Also, *Brabejum stellatifolium* will very likely be able to co-occur along with *Acacia mearnsii* in future drier conditions, as shown for two sites (Jonkershoek and Bainskloof). Still, *Acacia mearnsii* does have a strong drought-tolerance advantage in certain circumstances, in my case specifically, the high streamflow site. Its threat as an invader in Mediterranean climates (dry summers) becomes apparent as it clearly exhibited the lowest on average P_{50} values across all sites, and is thus more able to withstand drier conditions than the native species. Field xylem water potentials further support the contention that the invasive *Acacia mearnsii* has a significant advantage over native species under drier conditions.

Minimum field water potential (Ψ_{min}) in particular is an important measure of plant water status, as it represents the minimum moisture status plants must tolerate to remain physiologically active (Bashkar and Ackerly, 2006). My results indicated, as with vulnerability to cavitation, that within and between species and sites there were significant differences in water potentials at predawn and midday. For Mediterranean-type ecosystems, Bashkar et al. (2007) found an evolutionary link between minimum water potentials (midday) and drought-tolerant xylem, so one could expect plants experiencing low water potentials to select for more drought-tolerant xylem. However, comparing P_{50} values and midday water potentials revealed that the least drought-tolerant species, *Metrosideros angustifolia* shared the same water potential in response to water-stress conditions as the very drought-tolerant *Acacia mearnsii*. Midday water potentials for *Acacia mearnsii* ranged from -2.6 to -2.96 MPa, and its P_{50} values ranged from -3.7 to -4.43 MPa. *Acacia mearnsii* thus has a greater

safety margin against water potentials that would cause 50% of its xylem to cavitate (P_{50}) and so induce runaway cavitation. So, even though *Acacia mearnsii* had high predawn and midday water potentials, it balances this against having high drought-tolerance because of its greater safety margin against cavitation at the P_{50} level. *Brabejum stellatifolium* was shown to be as drought-tolerant as *Acacia mearnsii* in at least two sites, and experienced significantly higher minimum water potentials overall, ranging from -1.89 to -1.91 MPa. This species also never reached water potentials low enough to reach its P_{50} level, and is thus also operating at a greater safety margin against runaway cavitation. Moreover, *Brabejum stellatifolium* showed no significant difference in Ψ_{min} between sites. It is known that the major influence on Ψ_{min} measurements in plants is relative access to soil water (Ackerly, 2004; Wikberg and Ögren, 2004); one could thus deduce that *Brabejum stellatifolium* might more effectively access groundwater in diverse environments, whereas other species do not.

In contrast, *Metrosideros angustifolia* had minimum water potentials that ranged from -2.52 to -2.85 MPa, and its P_{50} values ranged between -1.42 to -2.59 MPa. *Metrosideros angustifolia* thus functions very close, and often beyond its P_{50} threshold. Especially at Jonkershoek this seemed to be the case, where it's P_{50} (-1.42 MPa) was far higher than its minimum water potential (-2.65 MPa). *Metrosideros angustifolia* showed the same trend in a previous riparian study done in multiple streams at Jonkershoek (Swift et al., 2008). The question is then – what other strategies might *Metrosideros angustifolia* use to tolerate or avoid drought, if any? It is also possible *Metrosideros angustifolia* closes its stomata during low midday water potentials, as was found for species that operate near their hydraulic limit or P_{50} (Linton et al., 1998; Wikberg and Ögren, 2004). Evidence for this as strategy against the onset of cavitation was obtained from delta ^{13}C isotope ($\delta^{13}C$) results.

$\delta^{13}C$ is seen as a biological expression of environmental conditions and provides an integrated measure of predicting availability of water to plant species (Stewart et al., 1995). Response to environmental conditions such as soil moisture is also species specific (Ponton et al., 2001; Akhter et al., 2005). At each site *Metrosideros angustifolia* exhibited the highest $\delta^{13}C$ values, and is therefore the most water use efficient of the three species. Akhter et al. (2005) showed that plants with a high WUE have the ability to maintain water uptake during drought by being hydraulically efficient through using a conservative water use strategy. This enables species to persist in a water-limited situation and can be seen as a form of drought adaptation. *Metrosideros angustifolia* therefore appears to adhere to a conservative water strategy as it was shown to be the most WUE species overall. The indication is that although *Metrosideros angustifolia* functions close to its P_{50} value, and also appeared to be the least drought-tolerant of the natives, it might still persist in future drier conditions due to a different

water use strategy, perhaps involving closing its stomata (isohydry) for some time during the day. However, this may induce mortality through carbon starvation as reserves of carbon are exhausted under extended drought conditions (McDowell et al., 2008). Interestingly, *Brabejum stellatifolium* and *Acacia mearnsii* showed no significant differences in WUE across all three sites. As stated earlier, these two species also did not differ significantly in terms of vulnerability to cavitation. Further research might reveal these two species probably having a more extravagant water use strategy in moist environments (Akhter et al., 2005), as was observed at DuToitskloof. The nature of *Acacia mearnsii* to form dense stands, in conjunction with having low WUE, will consequently have detrimental effects on streamflow quantity as stated earlier.

Another strategy to achieve drought-tolerance within species is where xylem cavitation proceeds, but just not through the whole plant (Rood et al., 2000). This is also known as hydraulic segmentation (Tyree et al., 1993), vulnerability segmentation (Tsuda and Tyree, 1997) or branch sacrifice (Rood et al., 2000). A study of riparian trees in semi-arid areas of North America (Rood et al., 2000), found that some branches on evergreen trees showed signs of senescence and die back. This form of drought adaptation was associated with vulnerability to cavitation, a phenomenon observed especially in riparian areas where streamflows had been reduced due to human interference (water extraction) (Rood et al., 2003). Fluctuation in access to groundwater is known to influence xylem anatomy (Shume et al., 2004). Field observations showed *Acacia mearnsii* and *Metrosideros angustifolia* to have signs of branch dieback whereas *Brabejum stellatifolium* does not (Pers. Obs.). Vulnerability curve slopes and their relationship with pit membrane structure (Figure 2.4) provide some evidence for branch sacrifice as an adaptation to drought conditions. For a given individual, vessels may vary in pit membrane size per branch, and are thus able to cavitate under different water potentials. Therefore some branches may reach P_{50} quicker than others, and therefore undergo die-back while the branches that had smaller pit sizes are still fully functional. Swift et al. (2008) also suggest that species such as *Metrosideros angustifolia* might utilize branch sacrifice as an adaptation against drought conditions. The interesting question is why species would have such a variable range of cavitation pressures as indicated by the slope of the curve. Species could select for optimal hydraulic performance in seasonally dry Mediterranean conditions, as drought-tolerance as a function of cavitation-resistant xylem is known to be hydraulically inefficient (Kolb and Sperry, 2004). Under mesic conditions, pit membranes are large enough to transport water efficiently through the xylem. When it is dry, those large porous pit membranes might cavitate, but not to the detriment of the whole plant

as not all plant organs contain that size pit throughout – resulting in branch sacrifice (Rood et al., 2000).

Analysis of curve slope data indicated that *Acacia mearnsii* and *Metrosideros angustifolia* exhibited a broad range of water potentials between P_{45} and P_{55}, in contrast to the significantly narrower range in *Brabejum stellatifolium*. This implies that *Brabejum stellatifolium* shows more or less uniform pit membrane sizes throughout each stem and makes the range of tensions it might cavitate under more predictable, whereas the other two species might be more difficult to predict due to variability in pit membrane size per branch (Pammenter and Vander Willigen, 1998). Although further empirical evidence is needed to substantiate such assumptions, *Acacia mearnsii* and *Metrosideros angustifolia* might experience hydraulic segmentation as an adaptation to drought-stress. Field water potential analysis showed that these two species experience higher midday drought-stress, and thus strengthens this argument. Branch sacrifice could also help explain the significantly weaker R^2 values recorded for *Metrosideros angustifolia* and *Acacia mearnsii* because, for a given water potential, one shoot could have lost more than 50% of conductivity, whereas another shoot could lose very little. However, although *Acacia mearnsii* and *Metrosideros angustifolia* showed variability in cavitation, *Acacia mearnsii* was still significantly more drought-tolerant than *Metrosideros angustifolia*. In accordance with the rare pit hypothesis (Christman et al., 2009), this might suggest that the size of that rare large pore in *Acacia mearnsii* is still smaller than for *Metrosideros angustifolia*, which could explain why it reached P_{50} at lower water potentials. So in a given stem for *Acacia mearnsii* and *Metrosideros angustifolia* there could be a combination of independently distributed larger and smaller pit membranes. Evidence from this study thus implies a link between pit membrane variability per stem and branch sacrifice as an adaptation for growing in mesic and xeric habitats, especially where there are predictable variation in dry and wet periods, as is the case for Mediterranean-type ecosystems (Médail, 2008).

Clearly *Brabejum stellatifolium* has a different strategy in drought-tolerance adaptation as opposed to the other species as it consistently showed a different trend in vulnerability curves and minimum water potentials. This species should persist in future drier conditions, and shows promise as a post-clearing restoration species. The indication is also that some species, like *Metrosideros angustifolia* in this case, might have another strategy towards responding to certain environmental conditions other than having a high safety margin against drought induced cavitation. It may add tremendous value to also investigate the hydraulic properties of seedlings and resprouts of the two native species and the invasive alien species to determine whether these trends holds true for the juvenile stage. This is

especially important as seedling recruitment may take place in microsites that may enhance existing dry conditions (e.g. sandbars) and may subject seedlings to drought-stress at an early stage (Mahall et al., 2009). This may contrast with resprouts as existing roots may give this type of juvenile a slight advantage. One would therefore expect that juveniles would be less drought-tolerant than the adults, but a study have shown that seedlings can be more resistant to drought-induced cavitation (Rice et al., 2004). In addition Linton et al. (1998) found no difference in vulnerability to cavitation between adults and juveniles. More insights on juvenile physiological responses to drought would improve restoration and replanting strategies by providing managers the ability to prioritise the more drought-tolerant species at the juvenile stage over those that are less sensitive.

A comparison of results over various streamflow regimes yielded interesting results, which fits in with the ecological strategies of the studied species. *Brabejum stellatifolium*, *Metrosideros angustifolia* and *Acacia mearnsii* (-3.87 MPa,-2.59 MPa and -4.43 MPa respectively) had their lowest P_{50} values at the intermediate flow site Bainskloof, suggesting that species here are more drought-tolerant compared to the other two sites. In contrast, Jonkershoek and DuToitskloof, respectively the lowest and highest flow sites, did not show apparent differences between species. Minimum water potentials (Ψ_{min}) per site also showed a strong influence on plant species selecting for more drought-tolerant xylem and supported Bainskloof as being the site where species experienced more stressed conditions during pre-dawn and midday. This coincides with intra-specific trends in drought-tolerance at Bainskloof. Additionally, Bainskloof was also the site where individuals had the highest WUE overall, which correlates with my water potential data, again indicating Bainskloof being a more drought-stressed environment. A similar result was found with *Vitis vinefera* where some sites were significantly more water-use efficient than others, promoted by a deficit in water availability (De Souza et al., 2005). There is strong evidence that small changes in environmental conditions induce selection of improved plant adaptation strategies with regards to safe and efficient hydraulic transport (Ponton et al., 2001).

This challenges the hypothesis that species will be more drought-tolerant at lower streamflow sites, as put forward by Swift et al., (2008) as well as other studies on riparian plants and their physiology (e.g. Willms et al., 1998; Rood et al., 2003). Assuming higher run-off depicts more plant water availability, may not accurately predict species drought-tolerance *in situ*. Microsite or other factors such as site specific geomorphology and microclimate might play a more prominent role in species selection towards more drought-tolerance (Hupp and Osterkamp, 1996). For example, in the current study, Bainskloof sample trees showed lower midday and predawn water potential as well as lower P_{50} values,

suggesting that these plants have less access to soil water. However, this site, according to its streamflow characteristics, was intermediate between the Jonkershoek and DuToitskloof sites. All three sites had highly permeable bed characteristics, though at Bainskloof the sample trees were found away from the water's edge, and grew in positions elevated above the stream, which may indicate less available groundwater, in contrast to the sample trees at Jonkershoek and at DuToitskloof. It may be possible that the roots of these trees may be spatially disconnected from the saturated zone during summer, when transpiration is highest. In general this will depress predawn and midday water potential, and will lead to some plants lowering their P_{50} values to adapt to these drier conditions, although this may depend on the ecological strategy of the species. In addition, at Bainskloof the valley is more incised than the two other valleys, the sampling sites are also found relatively higher in the valley (Mountain Stream Transitional as opposed to Foothills) (see Table 2.1) with lower potential for depositional processes, which may suggest lower groundwater reserves. However, this is very difficult to confirm as riparian environments are typically highly heterogeneous in space (Naiman et al., 2005).

2.5. Conclusions

Considerable variation existed within and between species in their vulnerability to stem cavitation as measured by vulnerability curves – an indication of species-specific responses to withstand drought stress (different life-history strategies), and the variation within species under different environmental conditions (Cruziat et al., 2002; Maherali et al., 2004). *Acacia mearnsii* was shown to be highly drought-tolerant, able to withstand low minimum water potentials and able to utilize more water, so it will be able to persist in future drier conditions, and must therefore remain a top priority to eradicate. Fortunately, *Brabejum stellatifolium* also appears likely to persist under future drier conditions. It is well suited for restoration, as also found by Swift et al. (2008). These consistent results between species and over sites strengthen the argument that this mechanistic approach to distinguish site-level drought-tolerance between plants and their drought-tolerance at a specific site, is a practical technique, with application in understanding future geographic distributions of species under climate change or their potential for use in restoration research. Vulnerability to cavitation and midday water potentials are also strongly linked for *Acacia mearnsii*, and therefore water potentials are a valuable tool to predict its plant functional strategy with regards to drought (Bashkar and Ackerly, 2006). More research in understanding the mechanism of plant

survival and mortality under different drought intensities and duration will be key in predicting species distribution and range for future drier conditions (McDowell et al., 2008).

Lastly, streamflow *per se* is not robust enough to use as a screening tool when predicting drought-tolerance of species at a specific site because there was no relationship between drought-tolerance and river flows. The management implications of this result are that Jonkershoek and DuToitskloof should be given top priority in clearing as both have shown less drought-tolerance of native species versus those from Bainskloof. However, specific microsite conditions are also a major consideration in prioritising sites. This is especially important when taking into consideration the projected rapid onset of drier conditions in the south-western Cape (New, 2002).

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Chapter 3

Relationship between Wood Anatomical Traits and Plant Response to Variable Streamflow in Native and Invasive Tree Species in Fynbos Riparian Ecotones

Abstract

The ecological integrity of riparian zones of the fynbos region in South Africa is increasingly threatened, especially by invasive alien plants (IAPs) such as Acacia mearnsii. *To explore whether variation in species-specific functional traits pertaining to drought-tolerance exists, I investigated possible differences in wood anatomical traits of key native species and the invasive* Acacia mearnsii *across three south-western Cape riparian systems that differ in streamflow quantity. This will add to our knowledge on different plant responses in changing environments. More specifically, how species-specific functional traits could contribute to the future persistence of* Acacia mearnsii *under predicted declines in streamflows. Wood anatomical traits such as wood density, vessel resistance against implosion, vessel lumen diameters and vessel wall thickness were measured. Wood density varied significantly between species, showing* Acacia mearnsii *having denser wood at sites in rivers with greater volumes of water. As higher wood density is indicative of increased drought-tolerance and typical of drier sites, this counter-intuitive response suggests that increased wood density was more closely related to midday water-stress, than streamflow quantity* per se. *Insignificant differences in wood density between sites suggest functional convergence in this trait across the studied riparian zones. Wood density also positively correlated with vessel resistance against implosion, additional evidence that* Acacia mearnsii *is more drought-tolerant than the studied native species. . The observed plastic response of* Acacia mearnsii *to variable water availability indicates that this species should persist under drier conditions, as projected for the fynbos region. Specific environmental factors that determine wood anatomy* in situ *are discussed, as well as a possible non-causal relationship between wood anatomy and drought-tolerance in these riparian systems.*

3.1. Introduction

Fynbos riparian zones of the south-western Cape are projected to endure significant future water shortages (New, 2002; Steynor et al., 2009). Much of this decrease in water quantity can be attributed to a synergistic relationship between increased water needs from a growing population (Davies and Day, 1998; Mukheibir and Sparks, 2005), drier average conditions due to global change (Schulze et al., 2001; New, 2002; Steynor et al., 2009), and finally the negative effect of invasive alien plants (IAPs) on streamflow quantity (Bosch and Hewlett, 1982; Le Maitre et al., 1996; Scott et al., 1998; Enright, 2000; Dye et al., 2001; Le Maitre et al., 2002; Dye and Jarmain, 2004). This projected decrease in water availability will likely have a direct and negative impact on crucial ecosystem services, but, it is clear that IAPs are a preventable stress. However, uncertainty exists surrounding IAPs and their possible distribution under future water limited scenarios, and how they will compete with native species under these conditions (Dukes and Mooney, 1999; Richardson and Van Wilgen, 2004). Therefore, knowledge of species-specific (native and alien) functional strategies relating to drought as a measure of determining possible niche differentiation and thus chances of co-existence (Ackerly, 2004), could add significantly to our knowledge on different plant responses in changing environment.

Plant functional traits are any attributes that could potentially influence the establishment, survival or fitness of a species, and are of great value in predicting different plants' responses to environmental changes (Reich et al., 2003). This is because functional traits in plant taxa may vary considerably within and between species (Reich et al., 2003). In woody species, there exists a trait trade-off between safety versus efficiency during xylem hydraulic transport (as reviewed in Chapter 1). Because the mechanical design of a plant is fundamental for survival and also directly influenced by the environment, wood anatomy is highly likely to be under strong selective pressure (Read and Stokes, 2006). Ackerly (2004) highlighted the value of investigating variation in plant functional traits, especially in a water limited scenario, to better understand how co-occurring species could react when disturbed. This approach provided crucial insights into the inter-specific variation that exists in communities under changing environmental conditions, and how wood anatomical properties contribute to this response. Essentially, trade-offs in functional traits are seen as a main driver of anatomical diversity (Sperry et al., 2006). This is why the study of xylem anatomical functional traits that may determine plant responses to dry conditions in space and time has become an increasingly important research topic (Read and Stokes, 2006).

Particularly the focus on how the hydraulic design of plants can satisfy conditions of safety and efficiency in space and time (Weitz et al., 2006).

Wood density, an important anatomical trait in tree species, is indicative of mechanical and physiological life history strategies and therefore a critical functional trait for understanding the evolution and ecology of whole plant performance in different environments (Swenson and Enquist, 2007). Wood density is also an important role-player in plant water transport (Wagner et al., 1998; Meinzer, 2003), and is thus responsive to environmental factors such as water availability and temperature (Hacke et al., 2001; Thomas et al., 2007, Martínez-Cabrera et al., 2009). Barij et al. (2007) showed how wood density in tree species increased with an increase in slope, where slope represented a water availability gradient (steeper gradient – less water available). They ascribed this phenomenon to xylem structure being influenced by *in situ* hydrological properties – the less water available, the more dense the wood (Preston et al., 2006). Mean annual precipitation (MAP) as a measure of water availability, was also shown to have a significant negative correlation with wood density, as shown for *Eucalyptus grandis* in warm temperate regions of South Africa (Naidoo et al., 2006). However, wood density could also be positively correlated with MAP (Wiemann and Williamson, 2002), and supported by Chave et al. (2006) who hypothesised that dense wood in wet Neotropical forests is a safety mechanism against invertebrate attack. Still, as safe hydraulic transport (selection against vulnerability to embolism) is known to be a crucial trait in plants, there is generally an important link between wood density and resistance against drought-induced cavitation of xylem under water-stressed conditions (Hacke and Sperry, 2001; Hacke et al., 2001; Meinzer, 2003; Wheeler et al., 2005; Jacobsen et al., 2006, 2007; Pratt et al., 2007). Less dense wood is seen as being more prone to cavitation, and as a consequence, high wood density is seen as indicative of resistance to drought-induced cavitation (Hacke et al., 2000; Hacke et al., 2001; Pratt et al., 2007). Drought stress could thus increase wood density as a preventative measure against hydraulic failure in water-stressed conditions (Stiller, 2009).

However, wood density needs to be examined in relation to its component parts (vessel traits), as density and vessel characteristics might represent two ecological axes, which can explain the variation that exists within communities (Preston et al., 2006). Three such vessel characteristics are lumen-to-wall ratios ($(t/b)^2$ – vessel mechanical resistance against implosion), vessel lumen diameter and vessel wall thickness. Density correlates significantly and positively with wall to lumen ratios $(t/b)^2$ (Hacke et al., 2001; Hacke and Sperry, 2001). So, plants that grow in places with lower water potentials require higher wall thickness (stronger walls) to resist implosion and drought-induced cavitation (Sperry et al., 2006).

Larger lumens, although hydraulically more efficient, are seen as indicative of less dense wood (Wagner et al., 1998; Tyree and Zimmerman, 2002). It is therefore helpful to seek ecological correlations between these components (among others) and wood density, as a method of investigating the link between wood density and mechanical strength against drought stress. Another valuable correlation with wood density is minimum water potential or midday water potential (Ψ_{min}). Minimum water potential (Ψ_{min}) is a good indicator of different plants' access to soil water (Ackerly 2004; Bashkar and Ackerly., 2006). Therefore if density values are correlated with the water potential where the plant is most stressed, one may find that plants that experience the highest amount of stress, due to very low water potentials, are likely to have denser wood (Ackerly, 2004; Jacobsen et al., 2006).

Wood density can thus be a valuable screening tool for identifying species that may be more vulnerable to variation in water availability in a specific community (Jacobsen et al., 2006). Of interest is how variable wood anatomy is for native and invasive species across a water availability gradient as hydrological properties are particularly influential on plasticity of plant traits (Valladares et al., 2007; Funk, 2008). A lack of variation (little or no plasticity) would suggest that species might endure significant stress under reduced water availability. IAPs in particular have displayed high trait plasticity in low resource environments, therefore differences in phenotypic plasticity between native and invasive species may provide insight into how co-occurring species could respond to changing environmental conditions (Funk, 2008). This can provide important knowledge into current and future geographic distributions of species – especially that of IAPs (Hulme, 2008). Literature supports the importance of density in hydraulic traits and thus also in determining distributions, so it would be instructive to see how one species can vary in adaptive strategy given a single variable – water availability.

The rationale of my study was therefore to investigate whether: (a) plastic responses in wood density occur in the IAP *Acacia mearnsii* (a major threat to functioning of riparian zones in the south-western Cape (Dye and Jarmain, 2004)) across a water availability gradient, and (b) how the wood density of co-occurring natives compares with that of the invasive species. This was done at fine resolution (a narrow geographic range with similar climatic regimes but different streamflows), where I investigated wood density and its components ($(t/b)^2$) and lumen and wall thickness diameters among three river systems in the Mediterranean-type fynbos, with streamflow volume as the surrogate for water availability (sensu Swift et al., 2008). Swift et al. (2008) found that streamflow might indicate plant water availability, with lower flowing streams accordingly having plants that had more drought-tolerant characteristics than those at higher flowing sites. I further investigated

whether *Acacia mearnsii* showed variation in wood density across a broad resolution (a wide geographic range with a marked gradient in rainfall), with mean annual precipitation as the surrogate for water availability; and in addition, how the wood density of a co-occurring native species within each site compare with *Acacia mearnsii* in wood density. Ultimately the following key questions were investigated:

1. At a narrow geographic range, how would the invasive *Acacia mearnsii* and two key native riparian tree species respond with regards to wood density across three sites that differ in streamflow (sensu Swift et al., 2008)? What do anatomical characteristics contribute?
2. At a wide geographic range, how plastic is *Acacia mearnsii* across sites with different precipitation, and within sites, how does its wood density compare to that of a co-occurring native species?

3.2. Methods

3.2.1. Description of Species and Study Sites

For the narrow geographic range study, I selected the native riparian species *Brabejum stellatifolium* (Proteaceae) and *Metrosideros angustifolia* (Myrtaceae), as these evergreen species, which are the only indigenous species of their genus in South Africa, are key species in south-western Cape Mediterranean-type riparian systems, and critical for replanting and restoration in degraded riparian ecotones (Prins et al., 2004; Galatowitsch and Richardson, 2005). The anatomy of the native species was also contrasted with the evergreen invasive woody species *Acacia mearnsii* (Fabaceae). Differing from the two natives, *Acacia mearnsii* can grow comfortably in upland areas under low water availability as it is not an obligate riparian species (in some parts of South Africa it is a forestry tree). The three species do, however, co-occur and compete in some degraded riparian zones.

The study sites were the Eerste River at Jonkershoek (33º57.621'S, 18º55.037'E; 200 m a.s.l.), Wit River at Bainskloof (33º34.217'S, 19º08.452'E; 279 m a.s.l.) and Moleanaars River at DuToitskloof (33º41.778'S, 19º13.263'E; 297 m a.s.l.). Site selection was based on the following criteria: the presence of the selected suite of native and invasive species; the river system is invaded and no restoration/clearing has occurred and sites differ in streamflow

quantity – i.e. ranging from high to low flow. Jonkershoek represents the low flow site, Bainskloof the intermediate flow site and DuToitskloof the high flow site (Figure 3.1).

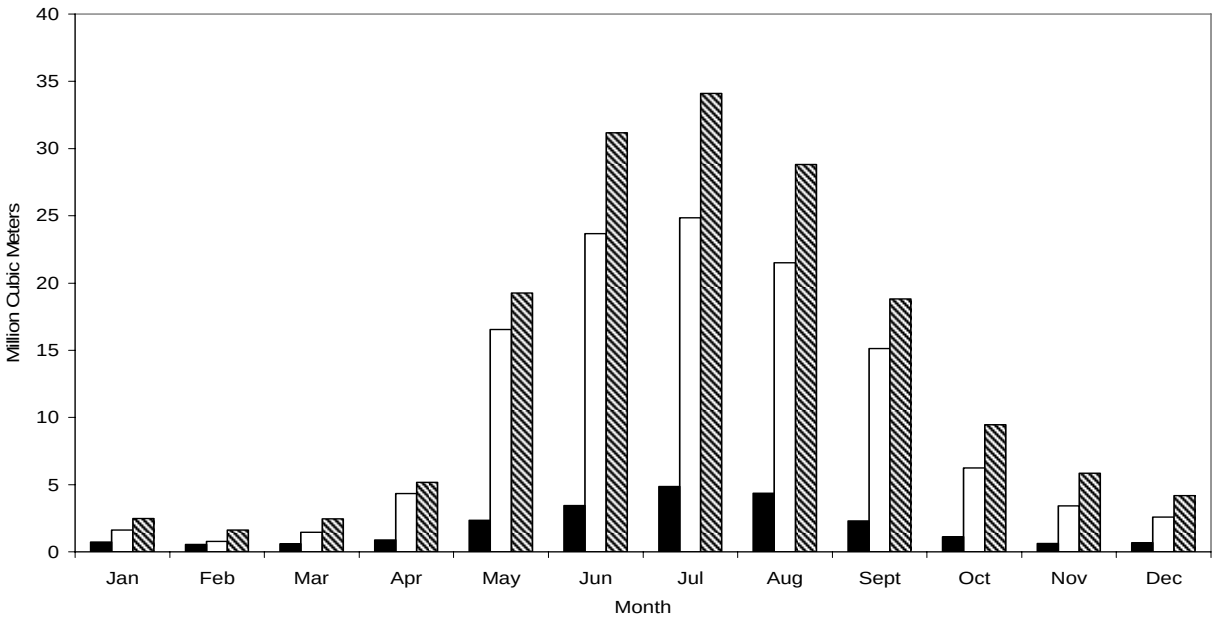

Figure 3.1. Mean monthly streamflow at the three fine-scale study sites representing low, intermediate and high streamflow regimes respectively (data from 1970 to 2008). Jonkershoek (black bars), Bainskloof (open bars) and DuToitskloof (cross-hatched bars). Data for Jonkershoek were obtained from the CSIR, and that of DuToitskloof and Bainskloof from Department of Water Affairs and Forestry (now Department of Water Affairs).

There are some complicating factors as far as streamflow data are concerned. The streamflow data shown for each of the rivers may be slightly biased by distance of the sampling sites to the location of the weirs, by the occurrence of woody alien species (*Acacia, Populus*, *Quercus, Eucalyptus* and *Pinus*) within the riparian zone, as well as other site specific factors. The reach where the study site in Jonkershoek is located is below the Kleinplaas Dam, operational since 1981, which regulates water flow close to the natural flow regimes. In addition, the catchment has been afforested since the 1920s (Bosch and Hewlett, 1982). This implies that current streamflow rates are in all likelihood significantly lower than what would have been found under prevailing natural conditions prior to 1900. Our sampling site at Jonkershoek was 2.4 km below the weir at Swartbrug, and the weir at Bosboukloof was 0.68 km away from the confluence with the Eerste River (data from both weirs combined to represend the streamflows of the Eerste River). It is conceivable that the streamflow measured may underestimate or overestimate streamflow depending on the relative contribution of shading of the stream, groundwater recharge and other sources of water to the stream. However, as Jonkershoek is the low flow site, this does not impact significantly on the choice and ranking of sites in terms of streamflow, as both DuToitskloof and Bainskloof

sites had notably higher streamflows (Figure 3.1). At both the latter sites, the sampling locations were also closer to the location of the weir (0.54 km upstream from the weir at Bainskloof and 5.7 km downstream from the weir at DuToitskloof).

Table 3.1 contains information on the river geomorphological characteristics. Jonkershoek and Bainskloof are draining catchments with predominantly sandstone geology, while at DuToitskloof is a mixture of sandstone and igneous rocks. The three sites occupy slightly different relative positions in the catchment: at Bainskloof the site is situated in the Mountain Stream Transitional zone, while the two other sites are in the Upper Foothills, which have a lower gradient. The sampled trees at the different sites also occupy different elevations above and distances away from the active channel (during low flow conditions). Jonkershoek catchment has been considerably modified over the last century, in contrast to the other two catchments, where streamflows reflect conditions close to the natural regime. This may impact long-lived vegetation at Jonkershoek.

For the wider geographic range study, I compared the wood density of *Acacia mearnsii* along six riparian systems that fell within regions that differed in mean annual precipitation (MAP) (Table 3.2). Using the MAP as the surrogate for water availability gradient, the sites were divided into two mesic, two semi-arid, and two coastal sites (Table 3.2). Within each site, I also compared wood density of the invasive woody tree *Acacia mearnsii* against one native co-occurring species, as listed in Table 3.2. The biogeographic differences between sites, necessitated the use of different native species at the different sites, lending the potential for direct comparisons. Native species selection within sites was therefore based on the most important species (aerial cover) co-occurring along with the IAP, even though none of the species are obligate riparian species, similar to the invader . This means that only *Acacia mearnsii* could be tested across the water availability gradient, whilst the native species were used to study differences in wood density within each site.

3.2.2. Wood Anatomical Properties

Wood density (D_t) was determined using the method as described in Hacke et al. (2000) and Pratt and Black (2006). The sample size was five individuals from each species. Stem segments were cut according to a standardized canopy position, compass direction and position on branch, to reduce possible within tree variation (Appendix B). Five to six stem segments of each individual tree were cut. Segments were then trimmed to around 2-3 cm.

Table 3.1. Site-specific information on each of the sites, including the major features of the rivers and the specific river reaches where the sites are situated, as well as pertinent information on the locality of the sample trees in relation to the stream

	Jonkershoek	**Bainskloof**	**DuToitskloof**
River	Eerste	Wit	Molenaars
River type (Naiman and Decamps, 1997)	Perennial	Perennial	Perennial
Geology	Sandstone	Sandstone	Sandstone and Igneous
Characteristic channel features (according to King and Shael, 2001)	Moderately steep, cobble-bed and mixed bedrock-cobble bed channel.	Moderately steep, dominated by bedrock or boulder	Moderately steep, cobble-bed and mixed bedrock-cobble bed channel
Longitudinal Zone (according to King and Shael, 2001)	Upper Foothills	Mountain Stream Transitional Zone	Upper Foothills
Range in the distance of sample trees from streams edge during low flow conditions	<2m	2-10m	<5
Zonation pattern where sample trees were located (Boucher and Tlale, 1999; Sieben and Reinecke, 2008)	Dry bank	Dry bank	Dry bank
Elevation above stream during low flow conditions	<1	1-3m	<1

Table 3.2. Description of broad scale study sites and species across which *Acacia mearnsii* was compared to with regards to xylem density over a mean annual precipitation environmental gradient

Site	MAP (mm)	Gradient Description	Native Species Compared	Latitude (S)	Longitude (E)	Elevation (m a.s.l.)
Eerste River, Stellenbosch	688	Mesic	*Brabejum stellatifolium* (Proteaceae)	33°56.266'	18° 53.260'	210
Witteklip River, Kareedouw	714	Mesic	*Cliffortia spp* (Rosaceae)	33°49.331'	24°25.723'	377
Buffeljags River, Buffeljagsrivier	551	Coastal	*Kiggelaria africana* (Achariaceae)	34°03.350'	20°32.343'	64
Buffeljags River, Heidelberg	413	Coastal	*Kiggelaria africana* (Achariaceae)	34°00.500'	20°46.614'	179
Keisie River, Montagu	334	Semi-Arid	*Psoralea pinnata* (Fabaceae)	33°41.232'	19°53.969'	789
Cango River, Oudtshoorn	241	Semi-Arid	*Noltea africana* (Rhamnaceae)	33°27.478'	22°20.711'	551

Stems were then dissected longitudinally so that the pith and bark could be removed. Segments were then immerged in water and degassed for 12-24h, or until no more air bubbles were observed. Fresh volume (xylem saturated with water after degassing) was determined using the water displacement method (Archimedes' principle) (Hacke et al., 2000). Dry weight was determined from measuring segments after dehydration in an oven at 75^{o}C for 48h. Density values were calculated by the ratio of dry weight to fresh volume (g/cm^3). Density data (at the broad and fine scale) were statistically compared using a factorial ANOVA followed by a Fisher LSD post-hoc test (Statistica Release 8, StatSoft Inc.) to identify any between site and species differences. At the wider geographic range, a one-way ANOVA was also used to compare *Acacia mearnsii* across the proposed environmental gradient. Also at the wider geographic range, the relationship between MAP and density for the IAP was analysed and explained by calculating the R^2 value. At the fine scale, multiple correlations were made between density and vessel properties.

Vessel properties were measured using methods described in Hacke et al., (2001) and Jacobsen et al. (2006). Stems segments were the same ones used in the vulnerability to cavitation experiment (Chapter 2). Transverse sections were shaved from one stem segment per individual, which were then mounted on a slide. Several images were taken of each transverse section with a digital camera (Leica DFC425, Leica Microsystems, Solms, Germany) attached to a light microscope (Leica DM1000, Leica Microsystems, Solms, Germany) (e.g. Appendix C). Images were analysed using the Leica Application Suite (LAS) software. In total, 100 measurements of vessel lumen diameter (*d*) and wall thickness were made per individual stem segment, rendering 500 measurements per species per site. As my main interest was wood specific density and not whole stem conductivity, I used the unweighted average vessel lumen diameter opposed to the hydraulically weighted average

diameter (Preston et al., 2006). The $(t/b)^2$, a function of two adjacent xylem conduits, where *t* is the length of both walls and *b* the length of the largest lumen, was obtained by measuring at least 20 adjacent conduits per individual, giving a total of 100 $(t/b)^2$ measurements per species per site. A factorial ANOVA, followed by a Fisher LSD post-hoc test (Statistica Release 8, StatSoft Inc.) was used to determine significant differences between species across different streamflow sites. To investigate any possible pattern in wood anatomical traits for tree species in the Mediterranean-climate south-western Cape riparian zones (e.g. if high wood density would also indicate high $(t/b)^2$ for species in these riparian zones, and how each species contribute to this relationship) (Iovi et al., 2009)) $(t/b)^2$, average lumen diameter, and wall thickness were correlated with density and analysed using R^2 values. Data were transformed as necessary to adhere to statistical models.

3.2.3. Xylem Water Potentials in the Field

For the fine scale study, stem xylem water potential was measured using a pressure chamber (Pockman et al., 1995) (PMS instruments, Model 1000, Oregon, USA). Measurements were made in summer (December-February, 2008-2009), when plants experience drought stress. Midday (12h00-14h00) measurements were made to obtain minimum (midday) water potentials (Ψ_{min}), and were carried out on the same individuals that were used for vulnerability curves measurements. Three healthy stems (with leaves) from each individual were cut and measured immediately on site. Three replicates of each treatment were made for each species across all sites. Data were analysed using a factorial ANOVA, to determine interactions between sites and species, followed by a Fisher LSD post-hoc test (Statistica Release 8, StatSoft Inc.). The relationship between Ψ_{min}, wood density and vessel properties was analysed using R^2 values.

3.3. Results

3.3.1. Narrow Geographic Range

Most species differed significantly in wood density across all sites ($P < 0.001$, Table 3.3), but the interaction of this trait between species and sites was insignificant ($P = 0.102$). *Brabejum stellatifolium* was the only species that did not differ in wood density across all sites; the other two species, *Metrosideros angustifolia* and *Acacia mearnsii*, presented

Table 3.3. Factorial ANOVA results for measured wood anatomical traits at the fine scale study. Values in bold indicate statistical significance.

Trait	Factor	*df*	F	P
Wood Density	Site	2	2.39	0.107
	Species	2	30.66	**0.000**
	Site*Species	4	2.10	0.102
Lumen Diameter	Site	2	11.6	**0.000**
	Species	2	61.0	**0.000**
	Site*Species	4	3.7	**0.005**
Wall Thickness	Site	2	2779.79	**0.000**
	Species	2	283.37	**0.000**
	Site*Species	4	166.63	**0.000**
$(t/b)^2$	Site	2	15.05	**0.000**
	Species	2	142.29	**0.000**
	Site*Species	4	3.65	**0.006**

Table 3.4. Mean values for wood anatomical traits measured across species and sites. Wood density values are in $g.cm^{-3}$, lumen diameter and wall thickness are in µm. Standard errors are given in parentheses. A factorial ANOVA was used to compare species and sites, with means separated using a Fisher LSD post-hoc test. Within and between sites, means with different superscripts differ significantly ($P < 0.05$). n = sampling size

Trait	Species	Jonkershoek	Bainskloof	DuToitskloof
Wood Density	*B. stellatifolium*	0.490^{a} (0.010)	0.487^{a} (0.027)	0.484^{a} (0.021)
(n = 30)	*M. angustifolia*	0.535^{ab} (0.015)	0.546^{b} (0.016)	0.528^{ab} (0.017)
	A. mearnsii	0.551^{b} (0.021)	0.641^{c} (0.022)	0.626^{c} (0.012)
Lumen Diameter	*B. stellatifolium*	39.63^{a} (0.46)	42.53^{b} (0.51)	42.48^{b} (0.57)
(n = 500)	*M. angustifolia*	38.85^{a} (0.39)	42.23^{b} (0.46)	41.78^{b} (0.52)
	A. mearnsii	46.03^{c} (0.65)	45.23^{c} (0.70)	46.15^{c} (0.60)
Wall Thickness	*B. stellatifolium*	2.89^{a} (0.033)	1.24^{b} (0.024)	1.23^{b} (0.012)
(n = 500)	*M. angustifolia*	1.80^{c} (0.024)	1.14^{df} (0.018)	1.13^{df} (0.015)
	A. mearnsii	2.69^{e} (0.041)	1.14^{f} (0.016)	1.33^{g} (0.016)
$(t/b)^2$	*B. stellatifolium*	0.0143^{a} (0.001)	0.0151^{ab} (0.001)	0.0176^{b} (0.001)
(n = 100)	*M. angustifolia*	0.0207^{c} (0.001)	0.0348^{df} (0.002)	0.0288^{e} (0.002)
	A. mearnsii	0.0291^{d} (0.001)	0.0372^{f} (0.002)	0.0352^{df} (0.001)

variable results across sites (Table 3.4). *Metrosideros angustifolia* was the only indigenous species whose wood density varied. It was significantly denser at the intermediate streamflow site (Bainskloof). In contrast, *Acacia mearnsii* consistently had significantly higher wood density compared to the native species at the intermediate streamflow (Bainskloof) and high

streamflow (DuToitskloof) sites. Within sites, the intermediate streamflow site (Bainskloof) was the only site where all the species differed significantly in wood density.

Lumen diameters differed significantly across species and sites ($P < 0.01$, Table 3.3). *Brabejum stellatifolium* and *Metrosideros angustifolia* showed a consistent trend in lumen diameter, with both species having significantly smaller diameters at the low streamflow site (Jonkershoek) as opposed to the other sites (Table 3.4). Within sites, lumen diameters did not differ between the native species. In contrast, *Acacia mearnsii* consistently had significantly larger lumen diameters than both native species across and within all sites. In addition, *Acacia mearnsii* did not differ in vessel lumen diameter between the sites.

Vessel wall thickness showed significant differences within and between sites ($P < 0.001$, Table 3.3). All species had their greatest wall thickness at the low streamflow site, Jonkershoek. All species also differed significantly in vessel wall thickness within each site. In addition, wall thickness of *Acacia mearnsii* differed significantly across all sites. *Metrosideros angustifolia* had on average the smallest wall thickness diameter (1.13-1.80 μm) and *Brabejum stellatifolium* on average the greatest (1.23-2.89 μm).

The ability of vessels to resist implosion $(t/b)^2$ varied significantly within and between sites ($P < 0.01$, Table 3.3). *Brabejum stellatifolium* had significantly lower $(t/b)^2$ ratios than the other species (0.0143-0.0176 μm; Table 3.4). *Acacia mearnsii* in turn had the highest $(t/b)^2$ ratios (0.0291-0.0372 μm). *Metrosideros angustifolia* was the only species that differed significantly in $(t/b)^2$ across all sites; the other two species, *Brabejum stellatifolium* and *Acacia mearnsii*, did not vary consistently across sites. Within sites, the low streamflow site (Jonkershoek) had the lowest $(t/b)^2$ values per species (0.0143-0.0291 μm) and the intermediate streamflow site (Bainskloof) the highest (0.0151-0.0372 μm, with the exception of *Brabejum stellatifolium*).

Measurements of wood density across all species and sites showed a significant and positive relationship with the ability of vessels to resist implosion or $(t/b)^2$ ($R^2 = 0.77$; $P < 0.005$) and lumen diameter ($R^2 = 0.47$; $P < 0.05$) (Figure 3.2). However, no relationship existed between wood density and wall thickness. The strongest correlation between wood density and its component anatomical traits was with $(t/b)^2$. Measurements of minimum water potential (Ψ_{min}) as an indicator of plant water-stress were significantly and negatively correlated with measurements of $(t/b)^2$ and wood density (Figure 3.3). The Ψ_{min} explained 80% of the variation in $(t/b)^2$, while it only explained 67% of the variation in wood density. All correlations showed species occupying different parts of the curve, indicating species-specific trends in the relationship between wood anatomical characteristics.

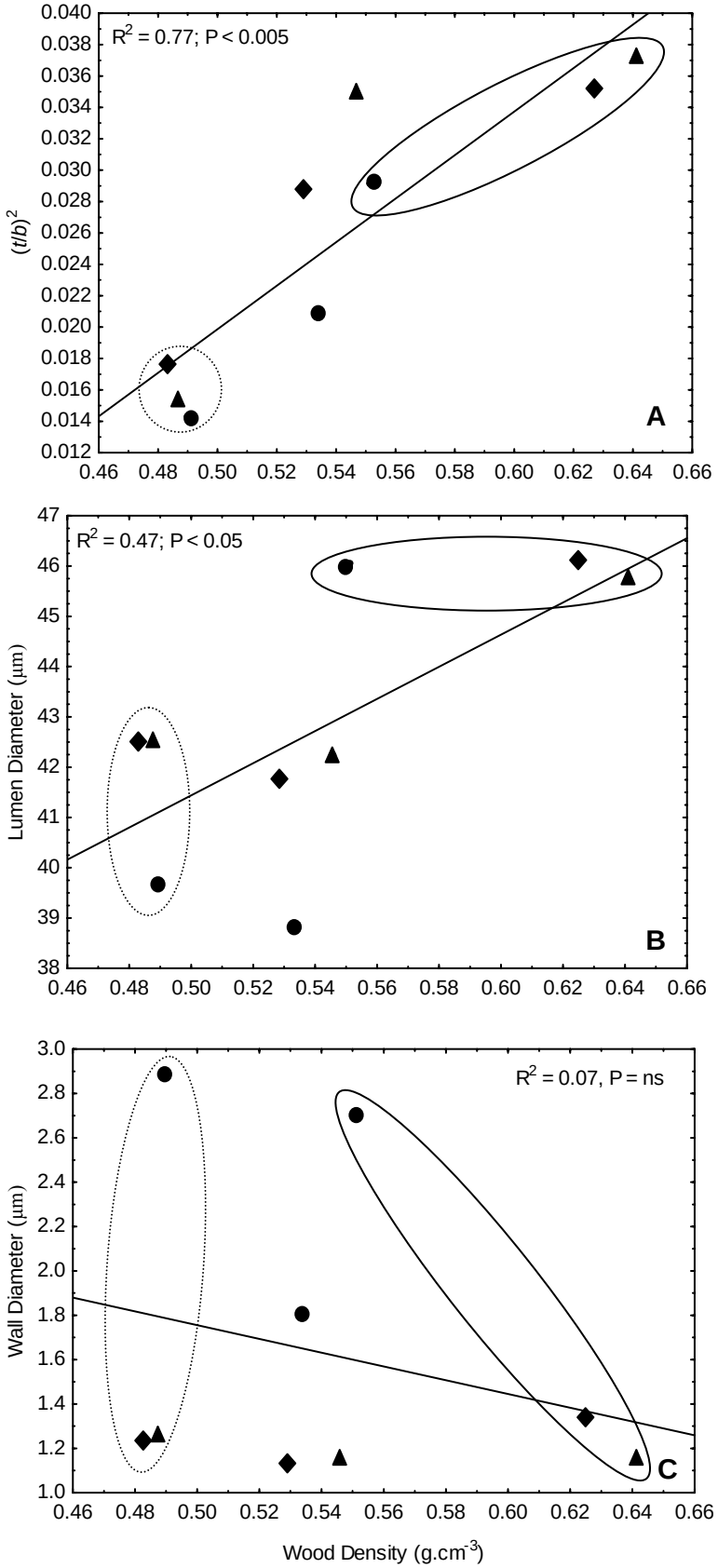

Figure 3.2. Plots of wood density versus (A) vessel resistance to implosion or $(t/b)^2$, (B) vessel lumen diameter, and (C) vessel wall diameter across all species and sites. In each graph the solid line shape represents *Acacia mearnsii*, the dotted line shape *Brabejum stellatifolium*, and open is *Metrosideros angustifolia*. The low streamflow site (Jonkershoek) is represented by circles, the intermediate streamflow site (Bainskloof) by triangles, and the high streamflow site (DuToitskloof) by diamonds.

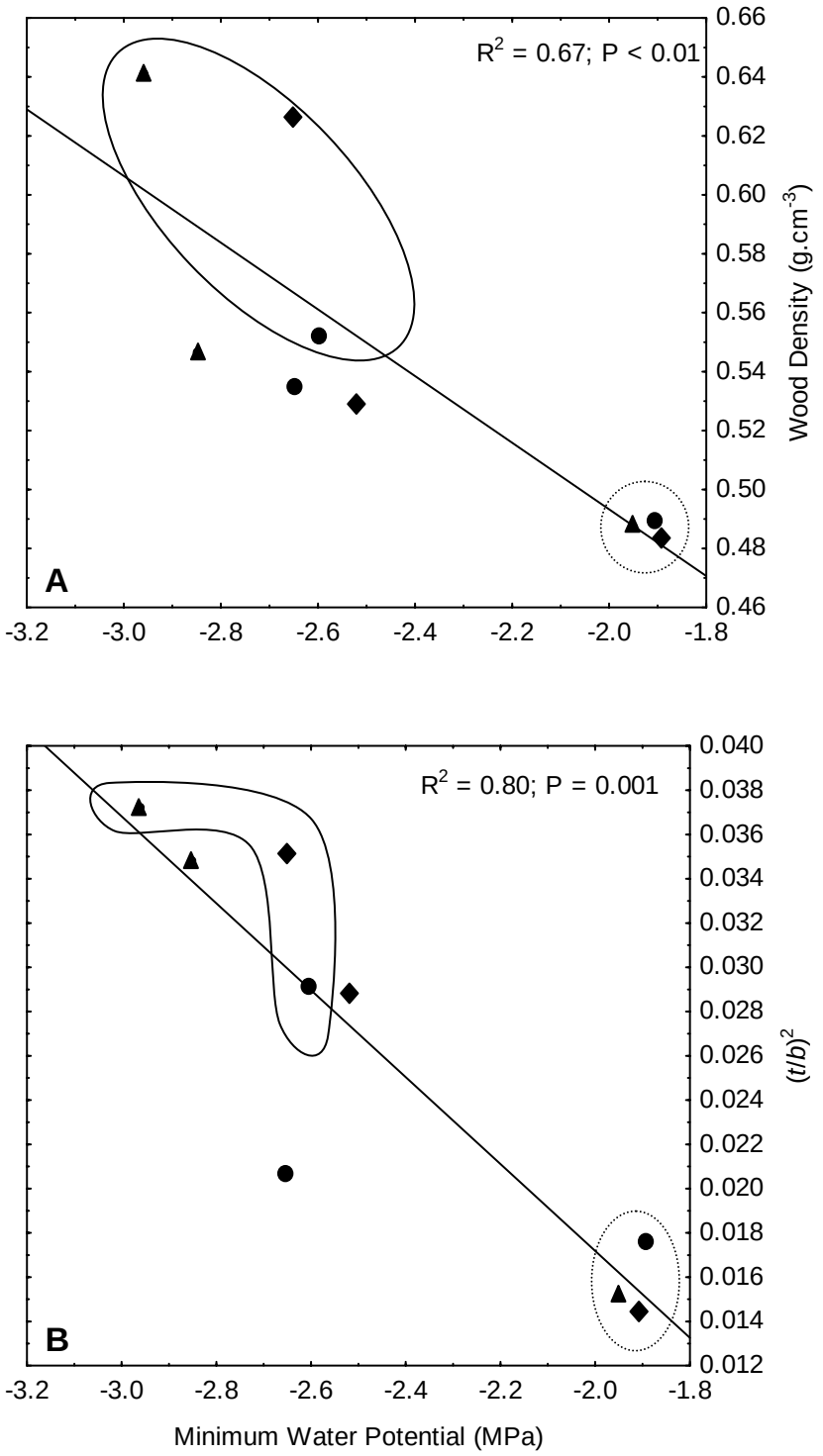

Figure 3.3. Minimum water potential (Ψ_{min}) as a function of (A) wood density and (B) vessel resistance to implosion or $(t/b)^2$ across all sites and species. In each graph the solid line shape represents *Acacia mearnsii*, the dotted line shape *Brabejum stellatifolium*, and open is *Metrosideros angustifolia*. The low streamflow site (Jonkershoek) is represented by circles, the intermediate streamflow site (Bainskloof) by triangles, and the high streamflow site (DuToitskloof) by diamonds.

3.3.2. Wide Geographic Scale

A one-way ANOVA of wood density of *Acacia mearnsii* across an environmental gradient showed significant intra-specific variation ($P < 0.001$, Figure 3.4). Wood density of *Acacia mearnsii* was significantly lower than the other species at the two mesic sites (0.487-0.511 $g.cm^{-3}$; $P < 0.05$), and on average the highest at the xeric sites, as well as one coastal site, Buffeljagsrivier (ranging between 0.572 and 0.593 $g.cm^{-3}$). Significant differences were observed between wood density of *Acacia mearnsii* and the selected co-occurring native species within sites ($P < 0.0001$; Table 3.5). Densities ranged between 0.451 and 0.564 $g.cm^{-3}$ for the native suite of species, and between 0.487 and 0.593 $g.cm^{-3}$ for the IAP (Table 3.6). A comparison between *Acacia mearnsii* and the chosen native species within each site, showed that *Acacia mearnsii* had denser wood at both xeric sites, and at one mesic site (Stellenbosch). *Acacia mearnsii* showed a negative relationship between MAP and wood density, but it was not significant ((although bordering significance at the 95 % level ($R^2 = 0.58$, $P = 0.08$; Figure 3.5)).

Table 3.5. Factorial ANOVA results for wood density of native and alien species combined in the wider geographic range study

Trait	Factor	*df*	F	P
Wood Density	Site	5	17.04	0.000
	Species	1	37.61	0.000
	Site*Species	5	17.61	0.000

Table 3.6. Mean values for wood density of *Acacia mearnsii* across an environmental gradient and between the selected native species (Table 3.2). All values are in $g.cm^{-3}$. Standard errors are given in parentheses. A factorial ANOVA was used to compare within and between species and sites, with means separated using a Fisher LSD post-hoc test. Within and between sites, means with different superscripts differ significantly ($P < 0.05$). n = 30

Site	Selected Native	*A. mearnsii*
Montagu (Xeric)	0.453^{a} (0.012)	0.593^{b} (0.017)
Oudtshoorn (Xeric)	0.511^{c} (0.005)	0.572^{bd} (0.010)
Buffeljagsrivier (Coastal)	0.542^{bd} (0.007)	0.589^{b} (0.012)
Heidelberg (Coastal)	0.564^{d} (0.010)	0.549^{d} (0.013)
Kareedouw (Mesic)	0.544^{d} (0.011)	0.487^{c} (0.008)
Stellenbosch (Mesic)	0.451^{a} (0.010)	0.511^{c} (0.011)

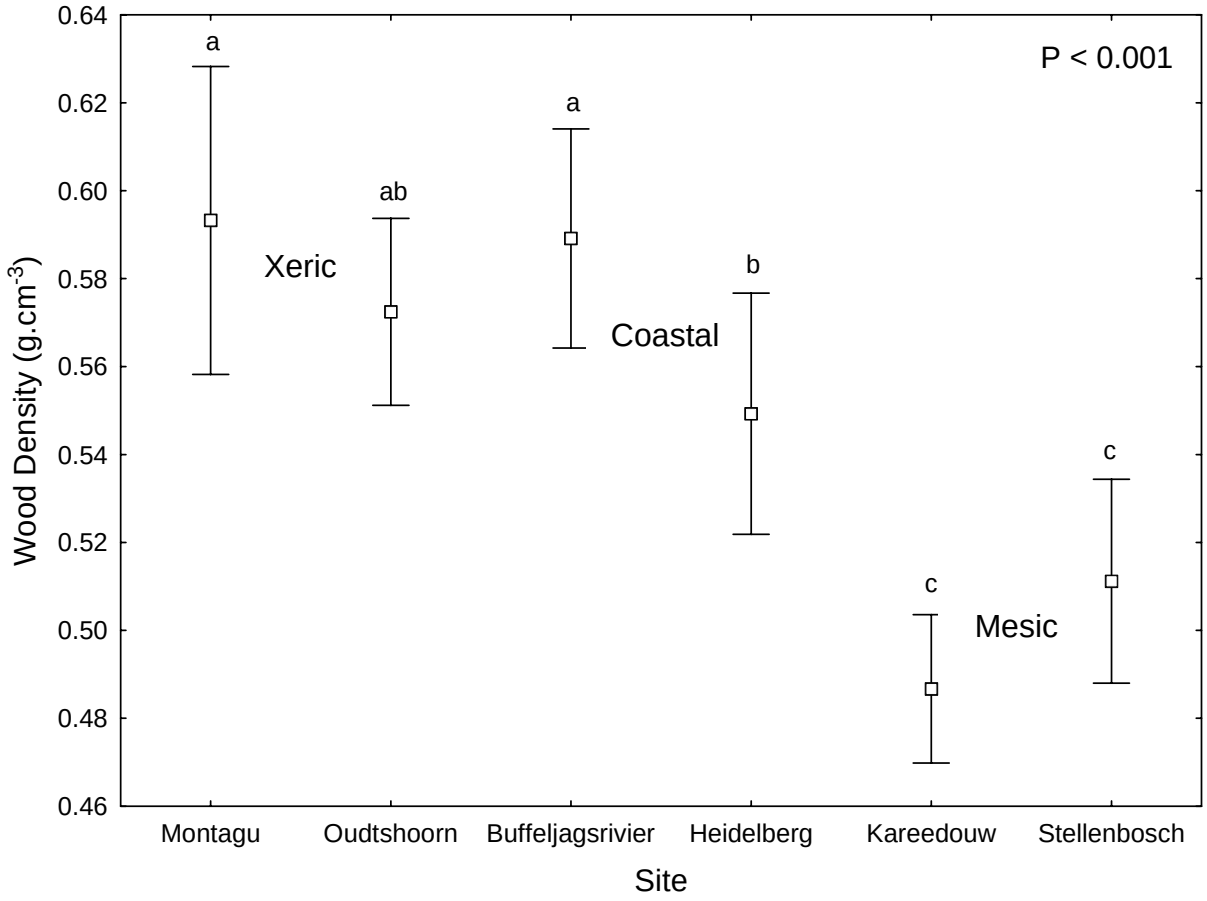

Figure 3.4. Mean wood density with 95 % confidence intervals for *Acacia mearnsii* across a water availability environmental gradient (as described in Table 3.2). A One-way ANOVA was used to compare density between sites, with means separated using a Fisher LSD test. Means with different superscripts differ significantly ($P < 0.05$). n = 30

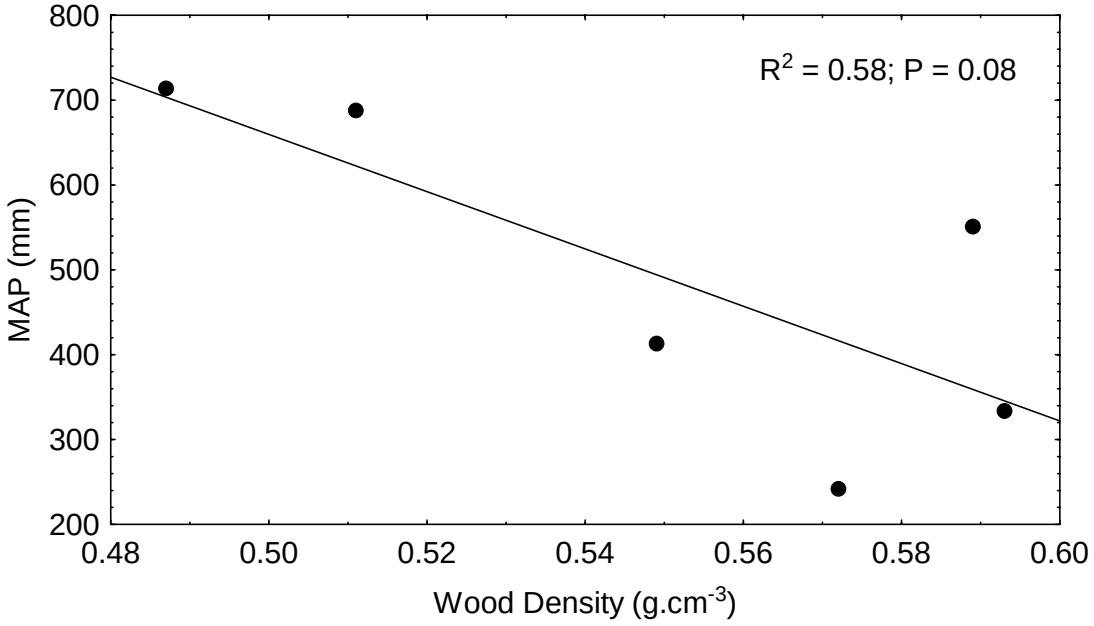

Figure 3.5. Relationship between mean wood density and mean annual precipitation (MAP) for *Acacia mearnsii*

3.4. Discussion

There is considerable phylogenetic variation in wood density (Chave et al., 2006; Read and Stokes, 2006). Variation observed within this trait is mainly attributed to the distribution of species in relation to water availability gradients (Ackerly, 2004; Preston et al., 2006, Poorter et al., 2009; Stiller, 2009). This suggests that plant traits generally will functionally converge towards having higher wood density in drier areas as a safety measure against drought-induced cavitation (Meinzer, 2003). However, we also know that plant responses to environmental stress can be highly dynamic and plastic within a species (Read and Stokes, 2006), which could allow some species to have an inherent advantage in certain circumstances, as seen for some Fabaceae IAPs in low resource environments (Funk, 2008). At the narrow geographic range, I examined how key fynbos riparian tree species co-occurring with *Acacia mearnsii* (Fabaceae) vary (if they do at all) in branch wood density across three sites where streamflow quantity was the proxy for water availability. Wood anatomical traits such as vessel mechanical resistance to prevent implosion $(t/b)^2$ and vessel lumen diameters and wall thickness were also assessed at this scale as they represent important components of wood density and thus have ecological relevance in trying to explain variation in density within and between species (Preston et al., 2006).

Inter-specific differences in wood density were significant ($P < 0.001$), with *Acacia mearnsii* showing significantly denser branch wood, at the two higher streamflow sites (Bainskloof and DuToitskloof). This is interesting as one expected species at Jonkershoek (the low streamflow site) to have the highest values. However, even if species-specific wood density showed considerable variation, wood density did not differ significantly between sites ($P = 0.107$). Clearly streamflow *per se* cannot be solely used to describe differences in wood density between species. This is in contrast to the hypothesis put forward by Swift et al. (2008) that increased streamflow is can be a surrogate for plant water availability. More evidence to strengthen this argument came from the relationship between wood density and Ψ_{min}. Minimum water potential (Ψ_{min}) had a significantly negative correlation with wood density, which is similar to what Jacobsen et al. (2006) found for evergreen shrubs in the Mediterranean-type climate region of South Africa. They found that plants differed significantly in their soil water utilization and, essentially, those that experience a greater seasonal water-stress will develop a greater wood density. In the Mediterranean-type climate of California, Preston et al. (2006) also argued that the best possible ecological correlate for wood density is the soil water content. It would therefore be instructive to incorporate Ψ_{min} when investigating a possible driver of wood density, as plants' relative access to soil water

(as measured by Ψ_{min}) (Ackerly, 2004; Bashkar and Ackerly, 2006) would be more accurate than correlations with superficial streamflow quantity, although the two might be interdependent. Also, not only are measurements like density and minimum water potentials relatively easy to obtain (Jacobsen et al., 2006), but also, when applied to the South African fynbos, which is predicted to undergo influential climate change, they could provide vital information on different drought adaptive abilities of species and thus provide insight on possible future distribution changes in response to reductions in water availability.

It is also imperative to correlate Ψ_{min} with site-specific geomorphological characteristics, as this may better explain what determines species responses towards being more drought-tolerant (Hupp and Osterkamp, 1996). In this study, the narrow geographic range study indicated that tree species at Bainskloof experienced drier environmental conditions, although it is the intermediate flow river. Therefore it could be that site-specific geomorphological characteristics might explain this observation better. Signs that this might be the case are indicated when referring back to Table 3.1, since Bainskloof occupy a different zone within the river (Mountain Transitional Zone), while both other sites are in the Upper Foothills. This means that the sampling site at Bainskloof had a steeper gradient than the other two sites, implying lower potential for depositional processes and therefore less soil to store water. Also, sample trees at Bainskloof were found away from the water's edge, and grew in positions elevated above the stream, which may indicate less available groundwater, in contrast to the sample trees at Jonkershoek and DuToitskloof. There are thus strong indications that plant access to groundwater might be more important to help explain plant functional strategies within a specific habitat or community. The importance of studying rooting depth and its relation to Ψ_{min} becomes apparent. More research needs to undertaken however to understand the micro-site conditions that may influence plant form and function in a water-stressed environment. This will have great application in optimising riparian restoration initiatives by identifying sites that are (and will be) experiencing greater future reductions in streamflows.

Subsequently, as greater wood density is seen as a broad indicator of adaptation to water-stressed conditions, it is clear that for these riparian systems, *Acacia mearnsii*, at least at two sites, might be better adapted against increases in droughts. Evidence for this came from correlating wood density with $(t/b)^2$, as these traits have been shown to be positively related, and would amount to species with high resistance against vessel implosion (less vulnerability to cavitation) having high $(t/b)^2$ values (Hacke et al., 2001, Pratt et al., 2007). The results of this study were consistent with the expected relationship ($R^2 = 0.77$), and consistent with the findings of Jacobsen et al. (2006). In addition, Ψ_{min} was also significantly

correlated with $(t/b)^2$. It is clear that there is a tight negative correlation between wood density and midday water potentials for species in the south-western Cape riparian zones. The influence of the environment on plant tissue level is thus linked to changes that promote drought-tolerance at the cellular level (Jacobsen et al., 2007, Pratt et al., 2007). It is also clear that this is a species-specific response because species clumped on different parts of the curves that explained the relationship between different functional traits (Iovi et al., 2009). It is therefore possible to determine different plant responses to water availability within a community, and in so doing be able to broadly predict drought-tolerance of species under climate change scenarios (*Acacia mearnsii* in this case) as argued by Jacobsen et al. (2006).

To further investigate whether we can broadly predict drought-tolerance of species using wood density of species, especially *Acacia mearnsii*, I asked whether geographically derived differences in wood density (using MAP as a proxy for plant water availability) existed within *Acacia mearnsii*. Also, how the wood density of *Acacia mearnsii* compares to that of a co-occurring native species, to see if functional trait convergence exists between species within a site. There was clear evidence that there were significant differences in wood density within *Acacia mearnsii* across sites (Figure 3.4), with mesic sites having less dense wood than xeric sites. As less dense wood is correlated with increased hydraulic conductance (Wagner et al., 1998; Stratton et al., 2000), these species thus show functional trait adaptive responses across a water availability gradient. The observed variation in response of *Acacia mearnsii* to drought was however not primarily influenced by MAP, as it showed a non-significant relationship between wood density and MAP. Still, the negative trend between wood density and MAP suggests that there is some interaction between these two factors. Therefore, adding more results from broader studies that specifically correlate species from very arid and wet regions, might help to strengthen the relationship between wood density and MAP (Martínez-Cabrera et al., 2009); especially since MAP may predict cavitation-resistance across broad geographic scales (Jacobsen et al., 2007). In contrast, Swenson and Enquist (2007) suggested that the evolution of wood density is mostly correlated with variation in temperature, and not necessarily MAP. Minimum water potential might also have a more prominent role in determining wood density, as suggested by the finer resolution study and literature. It is evident that the specific environmental factor that determines wood density is variable, and could be influenced by a combination of MAP, temperature and Ψ_{min}, depending on *in situ* microsite conditions. It would therefore be useful to test these factors in relation to wood density over a broad scale, to help refine possible variation observed within species between regions.

Additionally, comparing species within sites, *Acacia mearnsii* consistently showed higher on average wood density than the selected native species, but more importantly, had significantly denser wood at both xeric sites. Thus, in sites where plants have less access to soil moisture, this species should have an advantage in drought-tolerance compared to the studied native species. This is consistent to what was found at the narrow geographic range, and emphasises the invasive potential of *Acacia mearnsii* in the drier conditions projected by climate change models. The observed plastic response of *Acacia mearnsii* to variable water availability is very likely to have a positive influence on how these species respond to changes in hydrological and climatological conditions (Funk, 2008) projected for the fynbos region.

Interestingly, at the narrow geographic range, *Acacia mearnsii* also exhibited significantly larger lumen diameters and thinner walls compared to the native species, an indication that it might anatomically select for hydraulic efficiency rather than hydraulic safety through being drought-tolerant (Hargrave et al., 1994; Kolb and Sperry, 1999). Preston et al. (2006) found an inverse relationship between vessel lumen area and density, which they attributed to vessel lumen conductivity which increases with the fourth power of lumen diameter (Tyree and Zimmerman, 2002; Sperry et al., 2006), but results in less dense wood as the percentage of lumen area per transverse section of xylem is increased, thus limiting mechanical strength (Wagner et al., 1998). However, variation from this trend does exist, where vessel traits such as lumen diameters were found to be independent of xylem wood density (Martínez-Cabrera et al., 2009). In support of variation in the relationship between vessel traits and wood density, I found that lumen diameters were positively correlated with wood density. This suggests that species with denser wood would be less adapted to drought-induced cavitation, and better equipped at transporting water, a counter-intuitive response to the hydraulic safety versus efficiency relationship. In angiosperms, however, one cannot correlate conduit diameter *per se* against water-stress vulnerability as this was found to be a weak relationship (Hacke et al., 2000; Hacke and Sperry, 2001; Jacobsen et al., 2005). This is because water-stress vulnerability to cavitation is primarily influenced by the porosity of inter-conduit pit membranes (Hacke et al., 2000, Tyree and Zimmerman, 2002; Woodruff et al., 2008, Choat and Pittermann, 2009). Drought-tolerance might therefore depend more on pit membrane structure than vessel dimensions (which can be highly plastic) (Gorsuch et al., 2001). Also, Woodrum et al. (2003) found that for *Acer spp* in mesic environments (similar to the narrow geographic range study sites), there were no direct trade-offs between hydraulic conductivity and mechanical properties, as water transport at their study sites might not have been limited, and therefore had no selective influence on anatomy (Sperry et al., 2006). The

observed variation in vessel diameter and density at the narrow geographic range, thus adds to our understanding of plant strategies in mesic areas, and provides insight on how plants balance water availability, leaf evaporative demand and ultimately wood anatomical traits (Shume et al., 2004).

In addition, the spatial arrangement of xylem (i.e. whether xylem is clumped or solitary within a stem) in describing plant hydraulic strategy towards selecting either safety versus efficiency is currently poorly understood (Loepfe et al., 2007). Loepfe et al. (2007) modelled xylem spatial structure in an attempt to determine whether certain xylem topographies would indicate a more cavitation-resistant plant functional strategy. Their model suggests that increased clumping of vessels could mean a greater chance of having a neighbouring vessel filled with air, and therefore a greater chance of air-seeding and ultimately cavitation. Essentially greater vessel clumping could mean less resistance against cavitation. *Brabejum stellatifolium* had clumping of vessels, while both other species had a more classical solitary distribution of vessels within the fibre matrix (Appendix C). This is in contrast to the model of Loepfe et al. (2007), as *Brabejum stellatifolium* was significantly more cavitation-resistant than *Metrosideros angustifolia*, which had especially solitary distributed conduits. This adds to the current confusion of xylem networks and how it relates to plant hydraulic strategy.

3.5. Conclusion

At the narrow geographic range, wood density showed considerable variation between species, but to a lesser extent between sites, indicating functional convergence in this parameter of wood anatomy between taxonomically different species across these three riparian zones. However, *Acacia mearnsii* did have denser wood than the native species, particularly at the higher streamflow sites. Consequently, streamflow as an indicator of plant water availability and determinant of traits such as wood density is not accurate. Wood density should rather be seen in relationship with minimum water potentials, as sites that experienced more negative minimum water potentials had higher wood density. As wood density also positively correlated with vessel resistance against implosion $(t/b)^2$, it seems *Acacia mearnsii* is also the most drought-tolerant species within these species and across the studied riparian zones. These findings were also evident at the wider geographic range that accentuated the invasive potential of this species in a changing environment. At this scale, there were distinct differences in wood density between species (*Acacia mearnsii* versus the

native species within sites), and between sites (plastic response of *Acacia mearnsii* across an environmental gradient). These results support the argument that water availability has a strong selective effect on wood anatomy (Read and Stokes, 2006; Sperry et al, 2006). There is also a possible important relationship between environmental factors such as MAP and wood density. The use of wood density to screen drought-tolerance of all species in a community should be approached with caution, as there might be a non-causal relationship between greater wood density and greater resistance against drought-induced cavitation.

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Chapter 4

General Discussion and Conclusions:

Determining vulnerability of tree species in a changing environment – the contribution of plant physiology and wood anatomy

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4.1. Plant Functional Traits in a Water Limited Scenario

The rationale for this study was to gain knowledge about how vulnerable key native species, and a threatening invasive alien species, are towards streamflow variability in the Western Cape fynbos ecosystem. As discussed in Chapter 1, this ecosystem is predicted to be prone to future water deficits due to the synergistic impacts of various disturbances on water availability. I therefore set out to determine whether woody riparian plants show different physiological (Chapter 2) and wood anatomical hydraulic traits (Chapter 3) over a given water availability gradient. This was to gain a mechanistic understanding of how woody species, especially invasive species, adapt their hydraulic strategy to their environment and what this means for the possible future distribution of native and invasive species. This understanding can provide valuable insights into the use of plant functional traits as tools in prioritizing restoration initiatives.

4.1.1. Key Findings on Physiological Traits and its Contribution to Drought-tolerance

To determine possible species-specific responses to future water-limited scenarios, I asked how key native fynbos riparian woody tree species (*Brabejum stellatifolium* and *Metrosideros angustifolia*) would compare in vulnerability to drought-induced cavitation with the woody invasive tree *Acacia mearnsii*. In addition, I asked which one of the key native species would be most suited for restoration. The following key findings were deduced:

- There are consistent trends and species-specific responses to drought-induced cavitation, indicating the variation in life-history strategies between different taxa. In general, *Acacia mearnsii* showed lower P_{50} values across all sites, and is therefore more resistant to drought-induced cavitation than the native species.

- The consistent differences between species and over sites strengthen the argument that using a mechanistic approach to characterise site-level drought-tolerance between plants is a practical technique, with application in: (a) understanding future geographic distributions under climate change or (b) potential for use in restoration research.

- Being highly drought-tolerant, able to withstand low minimum water potentials and able to utilize more water, *Acacia mearnsii* is likely to persist in future drier conditions. It will continue to be an unnecessary strain on an already water-limited country, and must therefore remain top priority for eradication.

- *Brabejum stellatifolium* has a high safety margin against drought-induced cavitation, evidence that it may be able to persist under future drier conditions. When compared to the other species, it was clear that *Brabejum stellatifolium* has a different drought-tolerance strategy as it consistently showed a different trend in the vulnerability curves obtained and also showed consistently high minimum water potentials across all sites. It is well suited to restoration as it will be able to grow under drier conditions.

- Although *Metrosideros angustifolia* showed high vulnerability to drought-induced cavitation, there is an indication that this species might have another strategy in its response to certain environmental conditions other than having a large safety margin against drought-induced cavitation. This may involve regulating water usage through stomatal closure during peak midday water-stress hours (isohydric species).

- Vulnerability to cavitation and midday water potentials are also strongly linked for *Acacia mearnsii*, and therefore water potentials should be a valuable tool to predict its plant functional strategy with regards to drought.

To determine site-specific responses to changes in environmental conditions, I asked whether streamflow could be a robust screening tool when predicting which sites would contain species most vulnerable to cavitation, as one would expect species at high flow sites to be less drought-tolerant, than those in low flow areas. The following key findings were deduced:

- Streamflow *per se* was not robust enough as a screening tool in predicting drought-tolerance of species at a specific riparian zone, as there was no evidence that lower streamflow rivers were more likely to host more drought-tolerant individuals or populations. This is in contrast to that found by Swift et al. (2008), and suggests that using streamflow volume as a surrogate for plant water availability is not accurate across riparian systems in the south-western Cape. Riparian geomorphological characteristics such as slope and plant elevation above the groundwater table are more likely to influence plant form and function within a habitat or community.

- In this study, Jonkershoek (low streamflow site) and DuToitskloof (high streamflow site) should receive top priority in clearing as native species in both sites showed less drought-tolerance compared to those from Bainskloof (intermediate streamflow site). They are, thus, more vulnerable to declines in streamflow due to the synergistic impacts of social water use, invasive alien species and climate change.

4.1.2. Key Findings on Wood Anatomical Traits and its Relationship with Variable Water Availability

Here I investigated whether different adaptive responses exist in wood anatomical properties of the *Acacia mearnsii* across a water availability gradient, and how the wood density of co-occurring key native species compare to that of the invasive species. The aim was to gain insight into how co-occurring species might endure projected increases in water-stress, and if *Acacia mearnsii* will be better adapted to such droughts or not. This was done, first, on a narrow geographic range, where I investigated how wood density of *Acacia mearnsii* and two key native riparian tree species would vary across three sites that differed in streamflow, and what role, if any, anatomical characteristics would play. The following key findings were deduced:

- There was variation in wood density within species, but not between sites, indicating convergence in functional traits between taxonomically different species across these three riparian zones.

- *Acacia mearnsii* showed significantly denser wood than the native key species at the higher streamflow sites. This counter-intuitive response may mean that using

streamflow as an indicator of plant water availability (sensu Swift et al., 2008) and thus wood density is not accurate.

- Wood density appears to be related to minimum water potentials, as plants at sites that experienced more negative minimum water potentials (lower water potentials) had higher wood densities. This finding suggests that soil water regimes, and the plants' access to that water, play an important role in determining wood anatomical traits. The importance of taking into account site-specific geomorphological characteristics of the riparian system in explaining the observed trends in plant form and function is also accentuated.

- Wood density also positively correlated with vessel resistance against implosion. As *Acacia mearnsii* had the densest wood, it seems that it is the most drought-tolerant species across all the sites.

In addition, to investigate whether *Acacia mearnsii* also show variation in wood density across a geographic scale, I asked how plastic this functional trait of *Acacia mearnsii* would be across a wider geographic range, with mean annual precipitation as the water availability gradient. Also, within sites, how wood density of the IAP would compare to that of a co-occurring native species. The following key findings were deduced:

- Wood density in *Acacia mearnsii* varied significantly across the mean annual precipitation gradient, showing that this species has a plastic response to changing environmental conditions over a broad geographic scale. This indicates that it will persist under climate change.

- *Acacia mearnsii* also showed significant differences in wood density compared to a key native species within sites. This was especially evident at the xeric sites, indicating its superior drought-tolerance at water-stressed sites.

- Mean annual precipitation could not explain all the variation in wood density over the wider geographic scale, but a possible negative relation does exist. However, again micro-site conditions could be more important in explaining plant anatomical responses than superficial water availability gradients.

- The wider geographic range results thus supported findings from the narrow geographic range that *Acacia mearnsii* will have high invasive potential even in a changing environment.

4.1.3. Linking Physiological and Anatomical Traits

Both the cavitation-resistance and wood anatomical results were consistent in showing the significant variation that exists within and between species. These results strengthen the argument that water availability has a strong selective effect on wood anatomy of species and that minimum water potentials are more accurate in describing *in situ* hydrological conditions than streamflow (although they might be interdependent). Both approaches also highlighted the invasive potential of *Acacia mearnsii* under future reduced water regimes. It therefore seems that there could be a causal relationship between physiological and mechanical characteristics of xylem hydraulic transport. Does this mean it is correct to infer that the use of wood anatomical traits (which are often easier to measure) such as wood density and $(t/b)^2$, are reliable indicators of drought-tolerance of species across sites?

Studies comparing vulnerability to cavitation and wood anatomy across different species often report contradictory results, where wood anatomical characteristics did not always accurately represent the actual drought-tolerance (P_{50}'s) (Iovi et al., 2009; Martínez-Cabrera et al., 2009). Although significant correlations have been found between vulnerability to cavitation and wood anatomical traits (Cochard et al., 2008), it seems that the relationship between wood density and vulnerability to cavitation, in angiosperms at least, is only reliable for species in arid areas, where competition for water is of great importance (Hacke and Sperry, 2001; Sperry et al, 2006). Data from Hacke and Sperry (2001) showed an inflection point in the correlation between vulnerability to cavitation and wood density, indicating that only for species above a certain wood density (usually observed in xeric sites) there would be a stronger link between being more drought-tolerant with increased wood density. Also, vulnerability to drought-induced cavitation is primarily influenced by inter-conduit pit membrane structure (Choat et al., 2003; Choat and Pittermann, 2009; Christman et al., 2009). So, unless all the interrelated parameters have been incorporated into a study, which is often not feasible to due technological restrictions (specifically determining pit membrane characteristics) (Iovi et al., 2009), care must be taken when predicting drought-tolerance of species based on either wood density or $(t/b)^2$ alone (Pratt et al., 2007).

Consequently, it was essential to investigate whether the studied riparian species in the mesic south-western Cape riparian zones would display a causal or non-causal (having a weak or no relationship) relationship between physiology and anatomy. This has great relevance regarding accurate riparian management: if there is a non-causal relationship between physiology and anatomy between all species, one can construct a standard method of determining drought-tolerance by using an easy measurement tool such as wood density (Jacobsen et al., 2006). If not, then it would be imperative to have knowledge of the non-causal relationship between physiology and anatomy when selecting the best method to determining drought-tolerance between taxa within a site.

4.2. Plant Stem Physiology and Stem Wood Anatomy – Causal or Non-causal Relationship?

Since the same sites, species and individuals were used to determine vulnerability to cavitation and wood anatomical properties, it was possible to integrate both data sets and see if any possible relationship exists between stem hydraulic and stem mechanical traits for riparian species in the Mediterranean south-western Cape (Iovi et al., 2009). Vulnerability to cavitation (P_{50}'s) from Chapter 2 and wood density and $(t/b)^2$ from Chapter 3 for all species and sites were consequently compared (Figure 4.1). There was no relationship between vulnerability to cavitation and either wood density or $(t/b)^2$ for species in the fynbos riparian zones (Figure 4.1). This contradicts the results of other studies. Hacke and Sperry (2001) showed their $(t/b)^2$ values strongly correlates with P_{50} (across species and sites). They concluded that $(t/b)^2$ may therefore be an accurate anatomical predictor of cavitation-resistance. Pratt et al. (2007) also found significant correlations between wood density, $(t/b)^2$ and cavitation-resistance. However, they also concluded that the evolution of cavitation-resistance need not necessitate an increase in $(t/b)^2$. Consequently, the lack of a relationship between wood anatomy and physiology regarding resistance against drought-induced cavitation seen in this study, suggests that functional anatomical trait convergence regarding drought-tolerance between taxonomically different species does not always exist (Meinzer, 2003, Jacobsen et al., 2007). Moreover, it provides further support for complex relationships

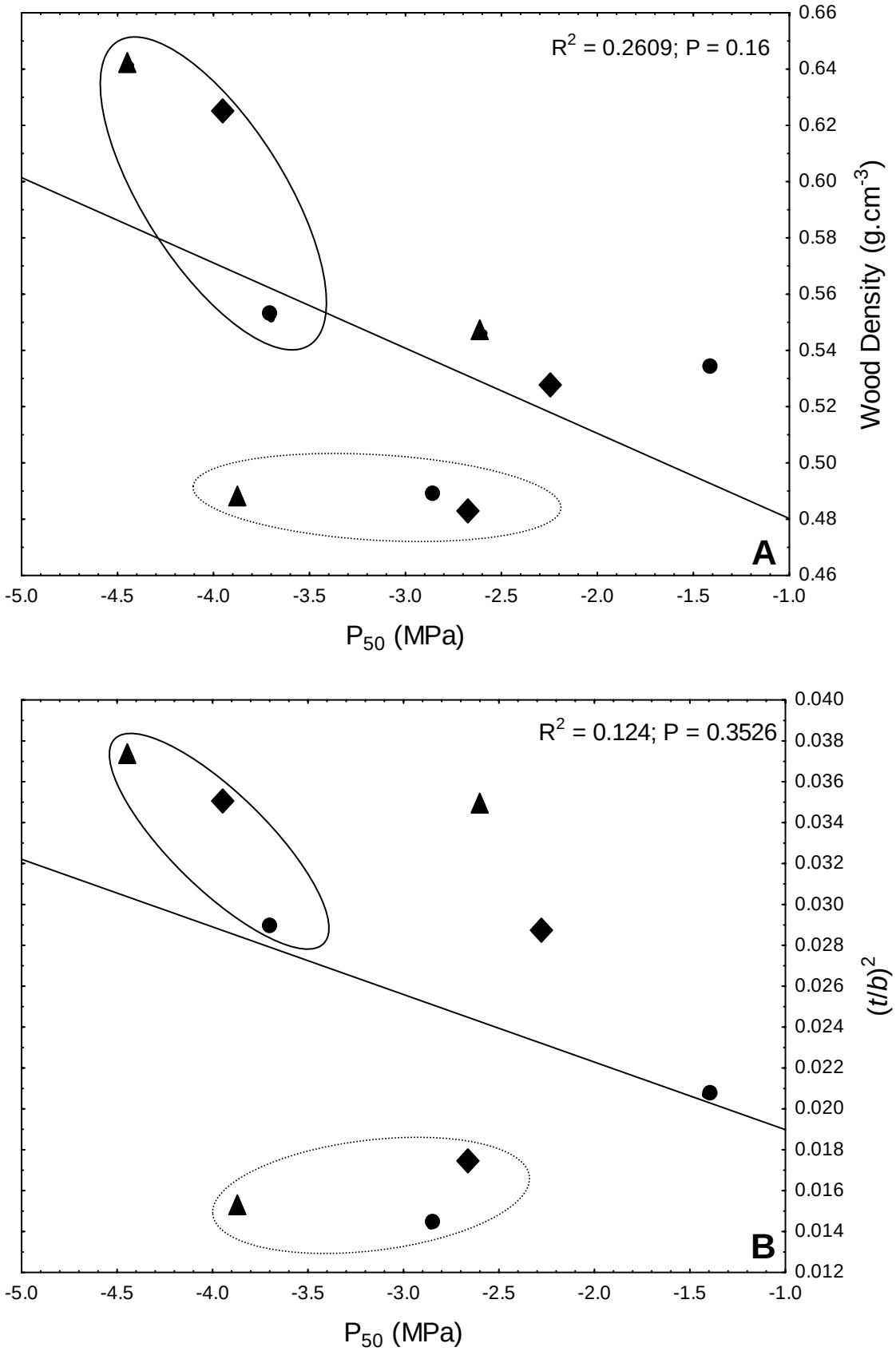

Figure 4.1. Drought-tolerance of all species across all sites, as measured by the water potential at which 50 % of xylem is cavitated (P_{50}), in relationship with (A) wood density, and (B) vessel resistance to implosion $(t/b)^2$. In each graph symbols surrounded by a solid line represents *Acacia mearnsii*, the dotted line *Brabejum stellatifolium*, and those in the open *Metrosideros angustifolia*. The low streamflow site (Jonkershoek) is represented by circles, the intermediate streamflow site (Bainskloof) by triangles, and the high streamflow site (DuToitskloof) by diamonds.

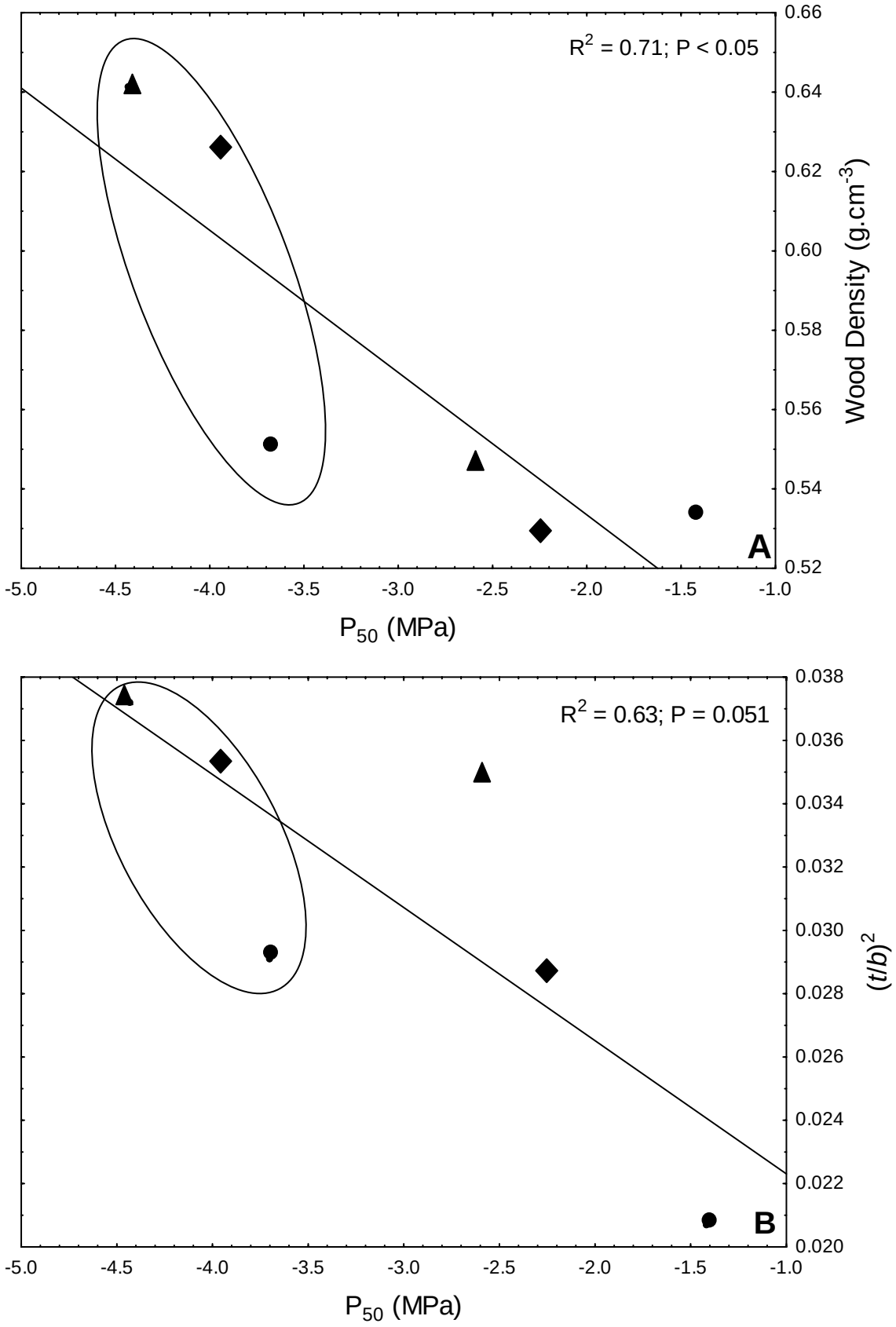

Figure 4.2. Drought-tolerance of *Acacia mearnsii* and *Metrosideros angustifolia* across all sites, as measured by the water potential at which 50 % of xylem is cavitated (P_{50}), in relationship with (A) wood density, and (B) vessel resistance to implosion $(t/b)^2$. In each graph symbols surrounded by a solid line represents *Acacia mearnsii*, and those in the open *Metrosideros angustifolia*. The low streamflow site (Jonkershoek) is represented by circles, the intermediate streamflow site (Bainskloof) by triangles, and the high streamflow site (DuToitskloof) by diamonds.

between certain wood anatomical traits and vulnerability to cavitation. These relationships will only become clearer when we increase the number of studies that compare intervessel pit membranes, other anatomical components and cavitation-resistance together (Jansen et al., 2009). Collectively, species-specific life-history strategies vary greatly in space and time, and it is apparent that very little is currently known about the genetic, physiological and biomechanical controls on plant hydraulic architecture (Read and Stokes, 2006; Weitz et al., 2006).

Evidently, different species could occupy different parts of a curve that describes the relationship between different plant functional traits (Iovi et al., 2009). In this study, *Brabejum stellatifolium* appears to be the main cause of the weak relationship seen in Figure 4.1. When this species is removed (Figure 4.2), the trade-off between wood anatomical traits and resistance against drought-induced cavitation becomes clearer. In Chapter 2 it was shown that *Brabejum stellatifolium* may have other strategies to avoid midday water-stressed conditions (as measured by minimum water potentials). As competition for water is a main driver of anatomy and vulnerability to cavitation, *Brabejum stellatifolium* may not experience water-stress in the same way as the other species. *Acacia mearnsii* has a strong relationship between high wood density, high $(t/b)^2$ and low vulnerability to cavitation, which is influenced by variations in water availability. This is in accordance with the results of my study, indicating its invasive potential in a changing environment. This is a prime example of the application of knowledge on species-specific life-history strategies when assessing different taxa across one parameter (e.g. water availability). It is therefore imperative that care must being taken when choosing a method to assess drought-tolerance between species as a possible non-causal relationship exists between resistance to drought-induced cavitation (P_{50}) and selected wood anatomical traits.

4.3. Recommendations for Assessing Drought-tolerance of Tree Species in South-Western Cape Riparian Zones

Due to the proposed non-causal relationship between drought-induced cavitation (P_{50}) and selected wood anatomical traits, the following recommendations for assessing drought-tolerance of south-western Cape riparian species are made:

- Using only wood density to assess drought-tolerance of species per site (e.g. Cochard et al., 2008) might not be accurate across all species as there does not seem to be a a strong relationship between wood anatomical traits and drought-induced cavitation.

- The drought-tolerance of *Acacia mearnsii* can be determined using either physiological or anatomical traits. This is very useful when assessing the invasive potential of this species across broad geographic areas. Technically, easy measurements such as wood density and minimum water potentials should provide reliable indications of its adaptive abilities pertaining to water-stress within and between sites.

- For the native species studied, the vulnerability curves provided a more reliable assessment of the differences in drought-tolerance across sites varying in water availability. Although vulnerability curves are not always easy to obtain because of the logistical complexities, the recommendations is to try to acquire P_{50} values first (as these values are seen as the most important parameter in determining drought-tolerance in trees). Once these measurements are obtained, further comparisons with wood anatomical traits can be made to ease future drought-tolerance assessments of species within or between communities or habitats.

In conclusion, mechanistic approaches to assess invasion potential of species and to improve species selection for restoration initiatives are very practical and of great potential value for protecting the integrity of south-western Cape river systems that are projected to endure severe water shortages. Research on ecophysiological and wood anatomical traits on key species provides valuable information about which species are vulnerable to projected climate changes, as well as insight into the best candidates for re-vegetation. This could then improve cost-benefit estimations of programmes such as Working for Water.

4.4. Future Research

Results from this study revealed important gaps that may add to our understanding of drought adaptation in woody tree species in a changing environment. The following areas need further investigation:

1. More research in understanding the mechanism of plant survival and mortality under different drought intensities and duration will be key in predicting species distribution and ranges for future drier conditions (McDowell et al., 2008). Measuring species stomatal conductance curves throughout the year would provide valuable insight on how species adapt under varying drought conditions. Determining whether plants are isohydric (maintaining transpiration under a degree of water-stress) or anisohydric (closing stomata when too water-stressed), could be used to assess which species will function better under extended droughts (usually isohydric plants), or which will function better under short intense droughts (usually anisohydric plants). Integrating findings of this nature with drought-tolerance as measured by vulnerability to cavitation would provide even more accurate results pertaining to prioritising sites for eradication at a broad geographical scale.

2. *Metrosideros angustifolia* does not function above its safety threshold as measured by vulnerability to cavitation. One explanation for this departure is that it may close its stomata during periods of water-stress, as seen with carbon isotope analysis, but there was an indication that it might also use vulnerability segmentation (Tyree et al., 1993) or branch sacrifice (Rood et al., 2000) as a form of drought adaptation. Further investigations into the occurrence of this phenomenon among key evergreen riparian species will add to our assessment of the probability of their persistence in water-stressed environments. This will help explain variations in adaptive strategies and why some species co-occur with species that were shown to be more drought-tolerant.

3. One key finding was the important role that minimum water potential has in selecting for certain physiological and anatomical responses in plants, rather than streamflow *per se*. *Brabejum stellatifolium* specifically showed a consistent trend in having high water potential that did not vary across different sites (where other species showed significant variation), a possible indication of differences in rooting depth (Hacke et al., 2000). Research on rooting depth variation within riparian zones differing in geomorphological characteristics for example, would provide answers to variation in water potentials, and could also be compared to vulnerability to cavitation (Jackson et al., 2000; Lopez et al., 2005, Jacobsen et al., 2007). Preston et al. (2006) also highlighted the importance of studying soil water content as a more accurate measure of plant water-stress within sites.

4. Porosity of bordered pit membrane structures in xylem is now widely accepted as being the underlying cause of cavitation in trees (Choat and Pittermann, 2009). Improving the link between plant physiological and anatomical traits to explain the hydraulic safety versus efficiency trade-off, implies having more research carried out on pit membrane structures across a wide variety of taxa and sites (Jensen et al., 2009).

5. It has been suggested that species may rely on larger vessels to facilitate better nutrient uptake (Gorsuch et al., 2001). Data on foliar nitrogen content of *Acacia mearnsii*, a potential nitrogen fixer (Burdon et al., 1999), versus the two native species studied (data not shown in this thesis), showed the IAP having three times more nitrogen (3%). Interestingly, *Acacia mearnsii* also showed significantly higher lumen diameters across all sites and species. This supports the contention by Gorsuch et al. (2001) that a link exists between vessel traits and nutrient uptake. Further research on the effect of nutrients on vessel properties may bring to light more knowledge on adaptation, and even invasion of species in nutrient-limited environments.

6. More research is needed at the critical juvenile stage to optimise restoration and replanting strategies and protocols. This may take the form of field sampling of juveniles of the three studied species (and other species) to determine hydraulic properties. Additionally greenhouse experiments under controlled environmental conditions would also be crucial in confirming observed trends in the relationships between physiological and wood anatomical hydraulic traits, and whether these are causal or non-causal. Greenhouses can also be used to test species across a wide variety of resource availability, providing more reliable predictions of responses in the field (Bradford et al., 2007; Stiller, 2009).

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Appendix A

Satellite Maps of Study Sites

Figure A1. Study sites at the wider geographic range, as used for wood density measurements (Chapter 3) (source: Google Maps)

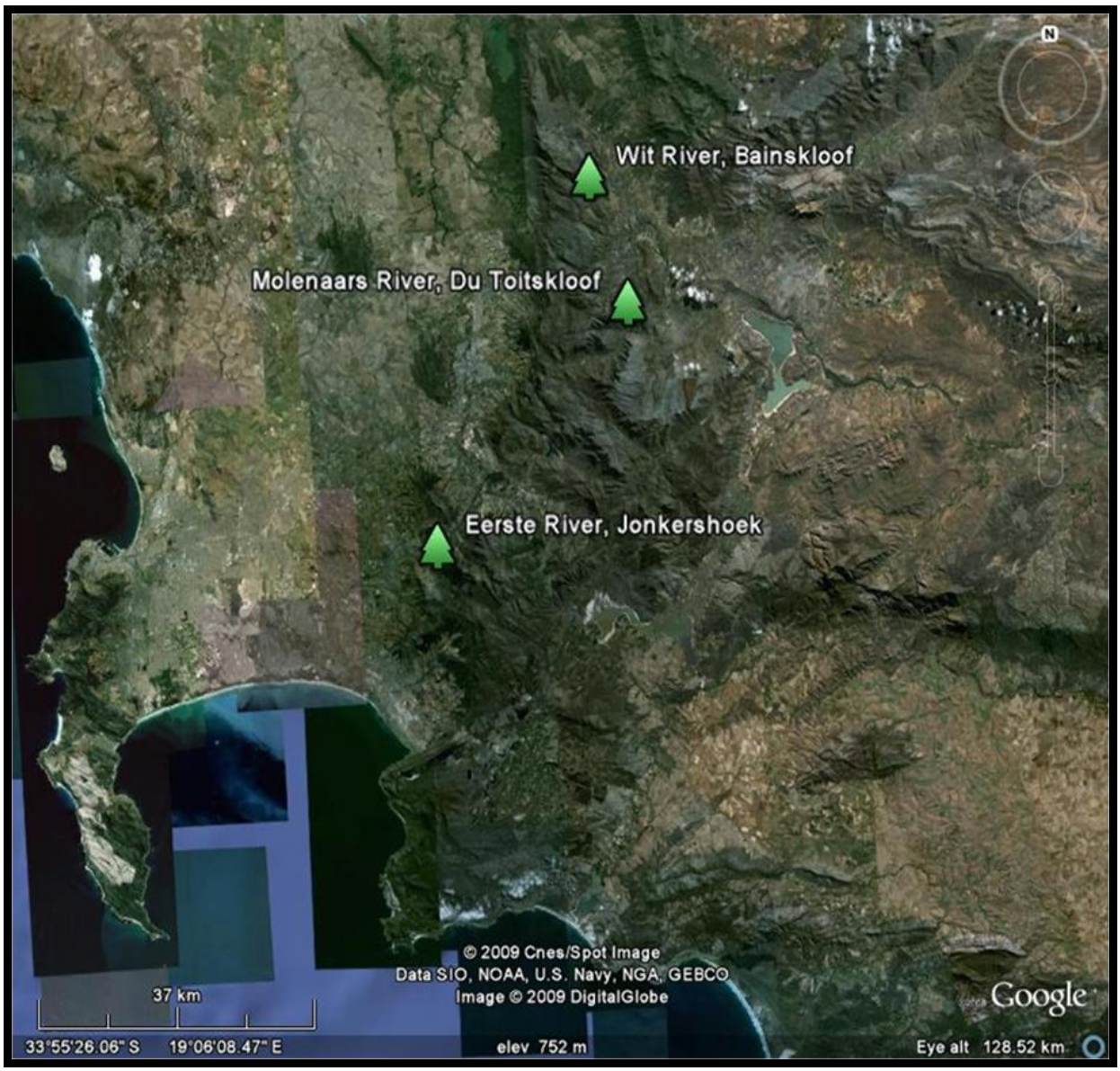

Figure A2. Study sites used for both the physiological (Chapter 2) and narrow geographic range wood anatomical measurements (Chapter 3) (source: Google Maps)

Appendix B

Standardizing Your Sampling Technique:

The question of in-tree variation

Introduction

Before sampling trees to determine wood density, the question arose whether variation in xylem wood density exists within a tree, and if one should therefore standardize the sampling method. Standardizing your sampling technique could decrease possible variation within a tree, and therefore decrease error in representation of differences within and between species across sites. I therefore asked: within a given tree, does stem wood density differ across compass direction (north or south facing side), canopy position (basal height versus top of tree), or branch node position (before or after first node)? Significant differences in either of the aforementioned criteria within a tree will thus lead to standardizing the sampling method throughout my study.

Methods

The pilot study was conducted in the Jonkershoek valley in Stellenbosch, South Africa (33º56.266'S, 18º53.260'E). The species chosen were *Acacia mearnsii* (Fabaceae) the invasive alien plant compared throughout my study, and *Brabejum stellatifolium* (Proteaceae), one native key tree species in my study. Density values were calculated by the ratio of dry weight to fresh volume (g/cm^3). Three individuals per species were selected and 40 measurements in total were made per individual: five measurements for each of the following criteria: South Top, South Basal, North Top, North Basal, replicated at the first and second node. Density within species was statistically compared using a factorial ANOVA (Statistica Release 8, StatSoft Inc.).

Results and Discussion

Table B1: Wood density values for *A. mearnsii* and *B. stellatifolium* across different sampling regimes. Density within species was statistically compared using a factorial ANOVA. All values are in $g.cm^{-3}$.

	A. mearnsii	*B. stellatifolium*
	P-values	P-values
Compass Direction	0.000112	n/s
Canopy Position	n/s	n/s
Node Position	n/s	n/s

According to this pilot study, the only variation in wood density values existed for *Acacia mearnsii*, particularly in the compass direction criteria. Although height (canopy position) and node position did not vary, it would still be instructive to set a sampling standard, to ensure any variation that might exist within some species, would be standardized throughout my study. Subsequently I sampled branches from basal height with a north facing compass direction, across all chosen species.

Appendix C

Microscope Pictures of Measured Wood Anatomical Traits

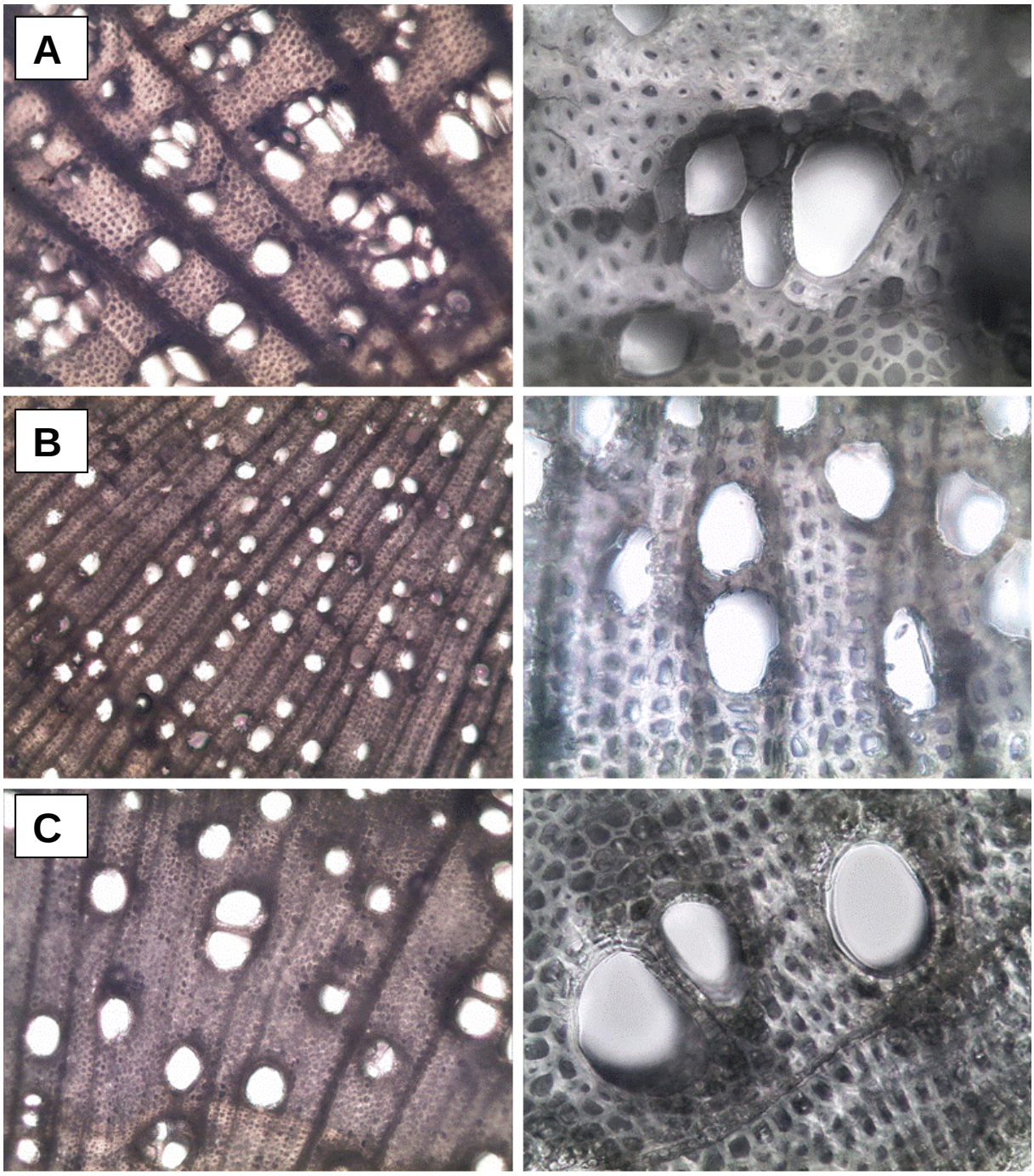

Figure C1. Samples of xylem cross-sections used for measuring wood anatomical traits of (A) *Brabejum stellatifolium*, (B) *Metrosideros angustifolia* and (C) *Acacia mearnsii*. For each species, the left panel is 100x magnification, and the right panel 400x magnification.